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WIDTH OF THE DENTAL ARCHES AT THE DECIDUOUS CANINES IN WHITE CHILDREN 4 TO 8 YEARS OF AGE

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INTRODUCTION

Width of the human dental arches in the region of the deciduous canine (cuspid) teeth has been studied using several terminal sites. These sites have varied from tip of cusp to gingival margin, from inner or lingual surface to outer or facial surface, and from contact area with the tooth ahead to contact area with the tooth behind. Specifically, bicanine (intercuspid) measurements have been determined as the transverse distance between (1) the most medial points on the erupted lingual surfaces of the canines (Campion, '20, Willett, '47), (2) the most medial points of the lingual surfaces at the gumline (Channing & Wissler, '08), (3) the midpoints of the lingual surfaces at the gingival margin (Henriques, '53), (4) the crests of the gum papillae separating the canines and lateral incisors (Schwarz, '25), (5) the centers of the canines near the cervical level (Stanton, Fish & Ashley-Montagu, '31), (6) the interdental gum crests, or contact regions, separating the canines and deciduous first molars (Lewis & Lehman, '29a, Sillman, '47), (7) the tips of the canine cusps (Cohen, '40, Burson, '52), (8) the most lateral points of the buccolabial surfaces of the canines at the gum margin (Spiller, '13, Colyer, '20), and (9) the most lateral points on the erupted portions of the canines (Chapman, '15, Northcroft, '22, Allen, '48). It will be recognized that in the orthodontically normal dental arch the smallest deciduous bicanine width is minimum interlingual distance (1) and the largest deciduous bicanine width is maximum straight-line distance between the buccolabial surfaces (9). In the present investigation original materials are reported for both of these dimensions.

In previous North American studies of deciduous bicanine diameter the data have been sometimes cross-sectional (Channing & Wissler,

'08, Henriques, '53), sometimes longitudinal (Cohen, '40, Burson, '52), and sometimes mixed (Lewis & Lehman, '29b, Alley, '34, Goldstein & Stanton, '35). The available analyses of longitudinal data that deal with age periods exceeding one year are based on samples of less than 30 subjects (Wallace, '27, Lancet, '30, Lewis, '36, Cohen, '40, Sillman, '47, Burson, '52). For the present investigation seriatim records covering a four-year span were amassed on 100 children.

Purpose

The major aims of this report are:

1. To present original findings for mean bicanine diameter at selected postnatal ages between 4 years and 8 years. Means are tabled for minimum and maximum distance between the deciduous canines of each dental arch on samples of North American white boys and girls studied longitudinally.

2. To describe variability in maximum bicanine diameter during the childhood period extending from 4 years to 8 years. Measures of absolute and relative dispersion are reported for twenty age-arch-sex subgroups. Graphic frames of reference serve to partition the normal range into five sections, and portray its change with age.

3. To depict increment distributions for maximum bicanine diameter representing the age intervals 4 to 6 years, 6 to 8 years, and 4 to 8 years. In each instance, distributions specific for sex and dental arch are characterized.

4. To explore a number of faciodental relationships involving maximum bicanine diameter. Pearson *r*'s are used to register the association between (a) maximum and minimum bicanine diameter, (b) maxillary and mandibular bicanine diameter, (c) size and increase in bicanine diameter, (d) bicanine diameter and distance between the deciduous second molars, (e) maxillary bicanine diameter and bizygomatic diameter, and (f) mandibular bicanine diameter and bigonial diameter.

5. To integrate the original results and the findings from previous North American studies on transverse diameter between the deciduous canines. Compactly constructed tables aid in colligating the assembled materials for mean size and change with age.

Subjects

The subjects were white children enrolled in the Facial Growth Study at the State University of Iowa. Their acceptance in this long-term research program was based upon willingness to participate, and likelihood of continuing residence in the Iowa City area.

With regard to ethnic background, the children were predominantly of northwest European ancestry. For more than 75 per cent, all four grandparents were immigrants or descendants of immigrants from the British Isles, Germany, Scandinavia, France, or the Netherlands; the remainder had at least two grandparents of northwest European lineage. Socioeconomically, almost 40 per cent of the fathers held professional positions and less than 10 per cent were unskilled workmen.

Use was made of a main sample and an enlarged sample. The foregoing description has characterized both. In the main sample there were 100 subjects with (a) maxillary and mandibular dental casts on file at annual ages from 4 to 8 years and (b) four deciduous canine teeth present at each age. The enlarged sample (employed to attain greater stability in the study of certain correlation problems) comprised 150 subjects with (a) maxillary and mandibular dental casts on file at age 5 years and (b) all deciduous second molars, first molars and canines present at this age. Every member of both samples met the further criteria: (c) surfaces needed in determining arch widths free from restorative dental work and (d) a record showing that neither dental arch had been subjected to orthodontic treatment. The last criterion connotes "normal dental arches" in the sense that orthodontic appraisal had not led to recommendation for treatment.

Subjects were examined at or near the anniversaries of their birthday, i.e., all of the children representing age 4 years were studied within a few days of this age, and so forth.

Procedures in Data Collection

The source materials for data on dental arch width were hydrocal casts made from alginate impressions. Special care was maintained in securing the impressions and in cast preparation. Occasional spot-checks on this effort to maximize the research usefulness of the casts were made, for individual children, by comparing averages derived from series of independent measures of the same dimension taken (a) directly in the mouth and (b) on the dental cast. The practical

equivalence of these averages showed that the casts closely approximated valid reproductions of the arches.

Two previous studies have dealt with the reliability of measurements of dental arch width taken on the Facial Growth Study casts (Meredith & Cox, '54, Meredith & Hopp, '56). These studies, utilizing both correlation and difference methods, analyzed series of measurements taken independently by two anthropometrists. Reliability coefficients homogeneous for age, sex, and dental arch were consistently above $r = 0.99$; standard deviations of differences were between 0.12 mm and 0.17 mm.

The arch width data of the present investigation have somewhat higher dependability. Using sliding vernier calipers, and reading to the nearest 0.1 mm, two trained anthropometrists independently measured each width. In instances where the two records did not differ by more than 0.2 mm, the average of the two became the datum that was used. In instances where the difference exceeded 0.2 mm, two additional independent measures were obtained and the average of the four became the datum.

The bicanine widths of each dental arch for which data were amassed are as follows:

1. Maximum diameter. The arms of the calipers were applied successively at a number of morphologically comparable points on the buccolabial surfaces of the deciduous canines until the greatest transverse distance was found. The measurement has been described aptly (Lancet, '30) as width between the outermost areas of the canines, both respective teeth being contacted at similar points.

2. Minimum diameter. This was determined as the shortest rectilinear distance between morphologically comparable points on the lingual surfaces of the deciduous canines.

These data were supplemented at one age (5 years) with data for maximum interbuccal width at the deciduous second molars, bizygomatic diameter, and bigonial diameter. Detailed descriptions of the collection procedure used in obtaining each type of material are available elsewhere (Meredith, '54, Meredith & Hopp, '56, Newman & Meredith, '56). It will suffice here to state that (a) the bimolar dimension was obtained on casts, the bizygomatic dimension by direct measurement, and the bigonial dimension from roentgenograms; (b) in each case measurement error was satisfactorily minimized; and

(c) all of the measuring instruments were subjected to frequent testing for control of systematic error.

Findings on Mean Bicanine Diameter

The means obtained for maximum and minimum distance between the deciduous canines of each dental arch are displayed in Table 1. This table supports the following findings:

1. Width of the dental arches in the region of the deciduous canine teeth increases with age. Generalizing for both diameters, both sexes and both arches, the average increase between 4 and 8 years of age is 2.6 mm.

TABLE 1

Dental Arch Width at the Deciduous Canines: Means (mm) for Maximum and Minimum Bicanine Diameter of Each Arch at Selected Childhood Ages.[1]

Age in Years	MAXILLARY ARCH				MANDIBULAR ARCH			
	Maximum Width		Minimum Width		Maximum Width		Minimum Width	
	Males	Females	Males	Females	Males	Females	Males	Females
4[2]	34.3	33.3	22.6	21.7	27.8	26.7	17.5	16.6
5	34.5	33.4			28.1	26.9		
6	35.1	33.9	23.5	22.4	28.8	27.5	18.5	17.3
7	36.0	34.8			29.8	28.5		
8	37.0	35.6	25.3	24.0	30.7	29.3	20.3	19.0

[1] Every mean is based on an N of 50; the subjects were 50 white children of each sex studied longitudinally.

[2] The reader may wish to interpret the sample means at a particular age as parameter estimates. For the maxillary arch, the standard errors of the mean approximate 0.3 mm (more precisely, they vary between 0.26 and 0.33 mm). For the mandibular arch the standard errors approximate 0.2 mm, varying between 0.19 and 0.22 mm.

2. The mean trend for deciduous bicanine diameter on age is curvilinear. In each arch-sex subgroup the central tendency values for maximum bicanine diameter manifest a slight rise (0.1-0.3 mm) from 4 to 5 years, a greater rise (0.5-0.7 mm) from 5 to 6 years, and a still greater rise (0.8-1.0 mm per year) from 6 to 8 years.

3. Transverse distance between the deciduous canine teeth is less in the average female than in the average male. Generalizing for both arches, both dimensions and all ages, the means for bicanine diameter on males exceed those on females by 1.2 mm. In each arch the sex difference becomes slightly larger with age.

4. Width of the maxillary dental arch in the region of the de-

ciduous canine teeth is greater than a corresponding width on the mandibular dental arch. Generalizing for both sexes and all ages studied, typically the mandibular arch is narrower than the maxillary arch by 6.4 mm in terms of maximum bicanine diameter, and by 5.1 mm in terms of minimum bicanine diameter.

5. On the whole, maximum deciduous bicanine diameter exceeds minimum deciduous bicanine diameter by 11.0 mm. The subgroup differences pertaining to the maxillary arch are larger than those pertaining to the mandibular arch by the average of 1.3 mm.

What further findings are accessible regarding average bicanine diameter in North American white children 4 to 8 years of age? Table 2 brings together, for the sexes pooled, means from the present study and from previous studies at ages 4 years, 6 years, and 8 years. Each of the investigations utilized is identified, and its sample delineated, in Table 3.

TABLE 2

Dental Arch Width at the Deciduous Canines: Means (mm) at Selected Childhood Ages from Studies on North American White Children of Both Sexes.

Study	4 Years N	4 Years Mean	6 Years N	6 Years Mean	8 Years N	8 Years Mean	Dental Arch Site
				Maxillary Arch			
1	100	22.2	100	22.9	100	24.7	Most medial point on canine
2					61	22.9	Most median point at gum level
3					80	24.3	Midpoint of lingual neckline
4[1]	69	27.2	66	27.9	52	29.4	Center of tooth, gingival plane
5[1]	91	27.2	81	27.9	57	29.7	Center of tooth, gingival plane
3					78	31.1	Lingual aspect of distal contact
6	64	28.7	19	28.5	11	31.1	Tip of canine cusp
7	28	28.9	28	29.8	28	31.6	Tip of canine cusp
1	100	33.8	100	34.5	100	36.3	Most lateral point on canine
				Mandibular Arch			
1	100	17.0	100	17.9	100	19.7	Most medial point on canine
4[1]	70	21.8	66	22.4	50	23.8	Center of tooth, gingival plane
5[1]	91	21.8	81	22.4	58	24.0	Center of tooth, gingival plane
7	28	22.8	28	23.8	28	25.6	Tip of canine cusp
8			24	23.7	24	25.8	Tip of canine cusp
6	66	25.7	18	25.4	11	27.2	Lingual aspect of distal contact
1	100	27.2	100	28.2	100	30.0	Most lateral point on canine

[1] Many of the same children were utilized in these two studies.

TABLE 3
Characteristics of the Samples on Which the Means in Table 2 Were Obtained.

Study	Investigator	City	Type of Study	Occlusion	Source of Data
1	Holcomb & Meredith, '56	Iowa City	Longitudinal	Untreated	Dental casts
2	Channing & Wissler, '08	New York (?)	Cross-sectional	Unselected[1]	Dental casts
3	Henriques, '53	Philadelphia	Cross-sectional	Unselected	Oral cavity
4	Alley, '34	New York	Mixed	Unselected	Dental casts
5	Goldstein & Stanton, '35	New York	Mixed	Unselected	Dental casts
6	Lewis & Lehman, '29	Detroit	Mixed	Normal	Oral cavity & dental casts
7	Cohen, '40	Minneapolis	Longitudinal	Normal	Dental casts
8	Burson, '52	Denver	Longitudinal	Abnormal[2]	Dental casts

[1] For those samples not selected with reference to dental occlusion, it is a reasonable assumption that few, if any, of the children had received orthodontic treatment.
[2] Angle classifications I and II.

Examination of Table 2 shows:

1. The available studies on North American white children 4 to 8 years of age are consistent in obtaining means for deciduous bicanine diameter that are (a) larger at age 8 years than age 4 years, and (b) larger on the maxillary arch than the mandibular arch. Those studies that analyzed their data according to sex (1, 2, 3, 5, 7, and 8) also concur in finding average bicanine diameter larger for males than females.

2. At each age, means for different deciduous bicanine dimensions ascend in the following order: distance between the lingual surfaces, distance between the centroid points (tooth centers at gum level), distance between the cusp tips, and distance between the buccolabial surfaces. In the maxillary arch, compared with minimum bicanine diameter (a) intercentroid diameter is larger by 5.0 mm, (b) intercusp diameter is larger by 6.5 mm, and (c) maximum diameter is larger by 11.6 mm. Corresponding average differences in the mandibular arch are 4.5 mm, 5.9 mm, and 10.3 mm, respectively.

3. Three studies provide means at age 8 years for distances between the lingual surfaces of the maxillary canines. All are based on samples of normal, North American school children. The mean of 22.9 mm from Channing & Wissler ('08) is conspicuously smaller than the other two. By statistical test, using the Channing-Wissler data and those of the present study, the hypothesis that the difference arises as chance variation from random sampling is rejectable at a high level of confidence ($t = 3.9$). A tenable explanation of the discrepancy has not been found.

4. At 6 and 8 years of age, means are reproduced from two investigations on transverse diameter between the cusp tips of the mandibular canines. In the one investigation the subjects were children with normal occlusion; in the other they were children with abnormal occlusion, i.e., Angle classifications I and II. It will be seen that corresponding means from these investigations are alike. Similar findings at age 8 years were obtained by Goldstein & Stanton ('38) on 19 boys with normal occlusion and 44 boys with abnormal occlusion. On each group the reported mean for intercentroid distance between the mandibular canines is 24.0 mm.

Findings on Variability in Bicanine Diameter

Variability was studied solely with reference to maximum bicanine diameter. Two approaches were made, one tabular and one graphic. Table 4 presents the standard deviation and coefficient of variation

TABLE 4

Dental Arch Width at the Deciduous Canines: Absolute (mm) and Relative (%) Variability Values[1] for Maximum Bicanine Diameter at Selected Ages.

Age in Years	MAXILLARY ARCH				MANDIBULAR ARCH			
	Males		Females		Males		Females	
	S.D.[2]	V[3]	S.D.	V	S.D.	V	S.D.	V
4	2.03[4]	5.9	1.82	5.5	1.32	4.7	1.38	5.2
5	2.06	6.0	1.81	5.4	1.34	4.8	1.43	5.3
6	2.15	6.1	1.88	5.5	1.46	5.1	1.51	5.5
7	2.24	6.2	2.02	5.8	1.55	5.2	1.57	5.5
8	2.30	6.2	2.09	5.9	1.59	5.2	1.49	5.1

[1] Every value is based on an N of 50; the subjects were 50 white children of each sex studied at successive annual intervals from 4 to 8 years of age.
[2] Unbiased estimate of the population standard deviation.
[3] Coefficient of variation.
[4] In each instance the standard error of the standard deviation is 0.1 x S.D. The same relation holds for the coefficient of variation, e.g., the standard error of 5.9 per cent is 0.59 per cent.

obtained on each of twenty age-arch-sex subgroups. Generalizations supported by this table are:

1. Maximum transverse distance between the deciduous canine teeth is more variable in the maxillary dental arch than in the mandibular dental arch. The maxillary age-sex subgroups are typified by a standard deviation of 2.0 mm and a variation coefficient of 5.9 per cent. Comparable typifying values for the mandibular arch are 1.5 mm and 5.2 per cent.

2. In neither dental arch are the obtained sex differences in variability significant statistically.

3. There is a slight increase in variability with age. Two tests of differences between correlated standard deviations were made, one on each dental arch. In both instances the difference evaluated was that between variability at age 4 years and variability at age 8 years for the sexes combined. The t values secured were 3.69 on the maxillary arch and 2.67 on the mandibular arch.

The initial step in developing the graphic description of variability was that of determining whether it could be assumed that the sub-

group distributions were obtained from statistically normal populations. Tests were made for skewness and kurtosis using the percentile method. The largest t values of the series were 1.20 for skewness and 1.49 for kurtosis.

On finding it was tenable to retain the hypothesis that bicanine diameter is normally distributed, each distribution was partitioned into five segments by calculating (a) the mean $\pm$ 0.52 S.D. and (b) the mean $\pm$ 1.65 S.D. These four values were chosen as corresponding to the 5th, 30th, 70th and 95th percentiles. Complemented by limiting values (the mean $\pm$ 3.0 S.D.), they supplied the ordinate data for plotting the graphs shown in Figure 1. It follows that for each graph, the segment designated "narrow" encompasses the lowest 5 per cent of the normal range, the segment designated "moderately narrow" comprises the next 25 per cent, and the succeeding segments include 40 per cent, 25 per cent, and 5 per cent, respectively.

The practical usefulness of Figure 1 to developmental anatomists and clinical odontologists (particularly orthodontists and pedodontists) is apparent. It provides a normative field for depicting individual status in bicanine diameter, specific for sex and dental arch, at any postnatal age between 4 years and 8 years.

Increment Findings for Bicanine Diameter

A number of investigators have obtained values on individuals and groups for increase or decrease with age in deciduous bicanine diameter. These values have supplied information on the average amount of growth over different age intervals and provided an indication of individual variation (see Table 5). The need for systematic analysis of the dispersion of increment distributions is clear.

Employing the basic data of the present study on maximum bicanine diameter, change in the width of each dental arch over three age intervals was determined on every subject of the main sample. For a particular interval, width at the beginning age was subtracted from width at the terminal age. The resulting values were arranged in eight ordered series, four for each arch. On a given arch the four series were for males 4 to 8 years, females 4 to 8 years, both sexes 4 to 6 years, and both sexes 6 to 8 years. Table 6 describes each ordered series in terms of its extreme values and four selected percentiles.

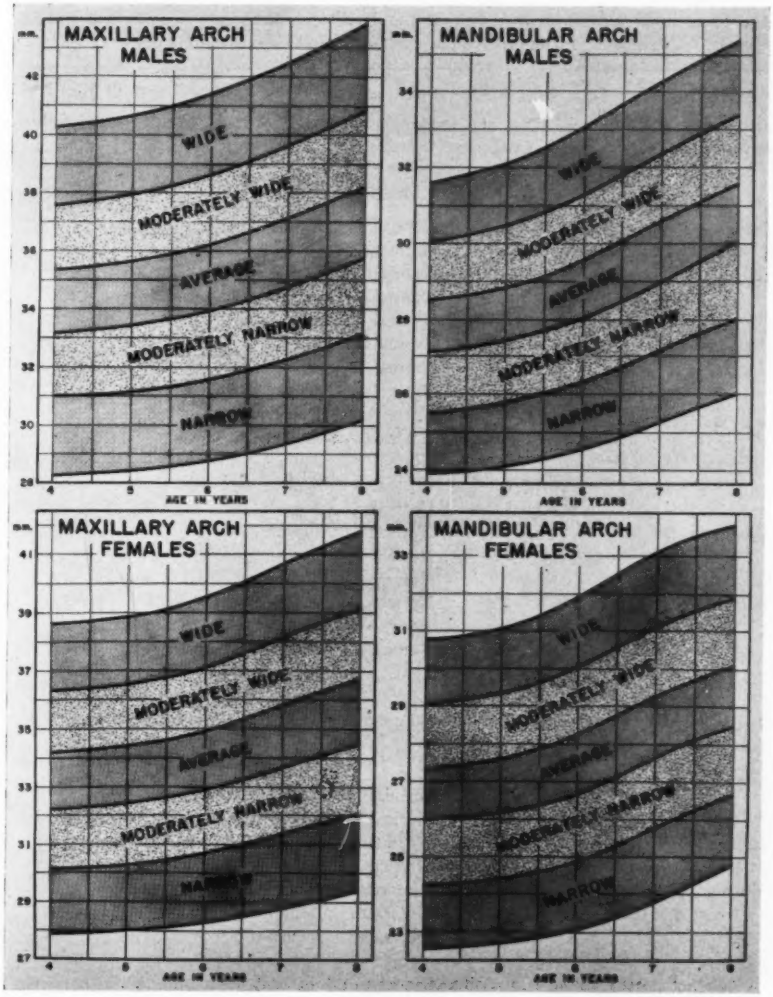

FIGURE 1
Frames of Reference for Maximum Width Between the Deciduous Canine Teeth.

TABLE 5

Age Changes in Dental Arch Width at the Deciduous Canines: Longitudinal Studies Reporting Increment Values (mm) for Individuals and Groups.

Investigator	Age Interval	Occlusion	Sex	Increase or Decrease	
				Upper Arch	Lower Arch
		Individuals			
Spiller, '13	7.5-8.5[1]	Normal	Male	0.2	..
Chapman, '15	4.0-9.2	Normal	Male	3.4	4.0
Colyer, '20	3.8-7.5	Normal	Female	2.9	2.0
	4.7-8.3	Normal	Female	2.5	2.3
	4.7-7.3	Normal	Male	2.3	1.4
Wallace, '27	3.5-8.2	Normal	Male	2.9	2.2
Lancet, '30	5.5-7.5	Untreated	Unknown	2.0	1.5
	6.0-7.5	Untreated	Unknown	1.4	—0.5
	7.0-8.0	Untreated	Unknown	3.4	1.9
	7.0-8.0	Untreated	Unknown	2.3	0.2
Schwarz, '33	7.0-9.0	Normal	Male	4.0	..
	4.0-7.0	Untreated	Male	3.5	..
	4.0-7.0	Untreated	Female	4.0	..
Chapman, '35	3.8-6.0	Normal	Female	0.5	0.7
	5.6-6.8	Normal	Female	2.8	..
Stanton, '35	4.8-6.9	Normal	Unknown	2.6	2.6
Sillman, '47	4.0-7.8	Normal	Male	3.9	3.3
	4.5-8.0	Normal	Male	2.0	3.1
	3.9-8.0	Normal	Female	3.9	4.1
	3.8-8.2	Poor	Male	2.2	2.6
	4.3-7.7	Poor	Male	3.0	2.0
	4.2-8.1	Poor	Male	2.2	3.8
		Groups[2]			
Goldstein & Stanton, '38	5.0-6.0	Normal	Both	0.4	0.5
	7.0-8.0	Normal	Both	1.0	0.8
Cohen, '40	4.0-6.0	Normal	Both	0.9	1.0
	6.0-8.0	Normal	Both	1.8	1.8
Burson, '52	5.0-8.0	Abnormal[3]	Both	..	2.7
Holcomb & Meredith, '56	4.0-8.0	Untreated	Males	2.7	2.8
	4.0-8.0	Untreated	Females	2.3	2.5

[1] Note that (a) the age intervals vary from 1 year to 5 years and (b) in a few instances the terminal ages are below 4 years and/or above 8 years.
[2] The N's for these groups, listed consecutively, are 61, 32, 28, 28, 24, 50 and 50.
[3] Fifty per cent Angle Class I, 50 per cent Angle Class II.

For the maxillary arch,

1. Increase in deciduous bicanine diameter from 4 to 6 years of age was less than 0.3 mm for 25 per cent of the subjects, between 0.3 mm and 1.1 mm for 50 per cent, and more than 1.1 mm for 25 per cent. While 75 per cent of the subjects gained 1.4 mm or more between 6 and 8 years of age, only 14 per cent made gains exceeding 1.4 mm in the preceding two-year period.

TABLE 6

Age Changes in Dental Arch Width at the Deciduous Canines: Percentiles (mm) Describing Distributions for Gain or Loss During Three Age Intervals.

Age Interval in Years	Sex[1]	Minimum	Percentiles 10	25	75	90	Maximum
			Maxillary Arch				
4-6	Both	—0.8	—0.2	0.3	1.1	1.8	2.6
6-8	Both	—0.1	0.8	1.4	2.3	2.9	3.7
4-8	Males	0.1	1.2	1.9	3.5	4.0	4.5
4-8	Females	0.2	0.8	1.6	3.1	3.7	4.1
			Mandibular Arch				
4-6	Both	—0.5	—0.1	0.3	1.3	1.9	2.9
6-8	Both	—0.4	0.9	1.4	2.4	2.9	3.9
4-8	Males	0.9	1.6	2.1	3.4	4.1	5.1
4-8	Females	1.0	1.5	2.0	3.1	3.7	4.2

[1] The 50 white subjects of each sex are considered jointly for the age intervals 4 to 6 years and 6 to 8 years, and separately for the age interval 4 to 8 years.

2. Ten per cent of the children had a slightly smaller bicanine diameter at age 6 years than at age 4 years. Dental cast examination revealed that none of these subjects lost deciduous incisors, or gave evidence of permanent incisor eruption, until after 6 years of age. For another ten per cent of the children (10 subjects), gain during the age interval 4 to 6 years ran as high as 1.8-2.6 mm. Regarding incisor status at the close of the biennium, it was found from cast inspection that 2 of this group showed deciduous incisors only, 7 showed one or both permanent central incisors erupting, and 1 showed four permanent incisors erupting.[1]

3. Increases with age in deciduous bicanine diameter over the

[1] Attention is called to the early paper by Colyer ('20) expressing the view that to some extent rapid increase in bicanine diameter is associated with eruption of the permanent incisors.

biennium 6 to 8 years were between 0.8 mm and 2.9 mm for 80 per cent of the subjects. Among the 10 subjects registering changes of less than 0.8 mm, 5 were found to show no oral eruption of permanent incisors and 5 to show four permanent incisors erupting. Among the 10 subjects gaining more than 2.9 mm, 4 showed one or two permanent incisors erupting and 6 showed three or four erupting.

4. There were individuals of both sexes for whom maxillary bicanine diameter was practically identical at ages 4 and 8 years. At the other extreme, there were individuals of both sexes for whom increase in maxillary bicanine diameter exceeded 4.0 mm. Generalizing with reference to sex, the chances are even that between ages 4 and 8 years a boy's increment in maxillary bicanine diameter will fall within the zone 1.9-3.5 mm and a girl's increment within the zone 1.6-3.1 mm.

Turning to the mandibular arch,

1. Table 6 shows that the distributions for change in bicanine diameter from 4 to 6 years and 6 to 8 years are similar to the corresponding distributions on the maxillary arch.

2. Without exception those subjects in the lowest one-tenth of the distribution for change in mandibular bicanine diameter between 4 and 6 years showed no loss of deciduous incisors or eruption of permanent incisors. The 10 subjects in the highest one-tenth of this distribution included 2 with deciduous incisors only, 5 with two permanent incisors erupting, and 3 with three or four permanent incisors erupting.

3. For the period 6 to 8 years, the casts of the 10 children who increased in bicanine diameter less than 0.9 mm registered two or three permanent incisors erupting in 3 instances, and four erupting in 7 instances. Nine of the 10 casts for children gaining more than 2.9 mm registered four permanent incisors in stages of eruption.

4. Gains in deciduous bicanine diameter over the age interval 4 to 8 years varied from 1.0 mm to 4.0 mm on females and 1.0 mm to 5.0 mm on males. These limits, specific for sex, may be considered to approximate the normal increment range, i.e., the range of natural (as distinguished from orthodontically induced) change in mandibular bicanine width.

The reader is referred to Goldstein & Stanton ('38) for increment information on bicanine diameter under different conditions of maloc-

clusion, and to Burson ('52) for greater detail regarding the influence of eruption of the permanent incisors on bicanine diameter.

Correlation Findings Involving Bicanine Diameter

Several associations pertaining to deciduous bicanine diameter were investigated. Included were five relationships between bicanine variables, and three relationships of bicanine width to other facial variables. In all instances, the assumptions for use of Pearson's product-moment formula r were satisfied. The correlation coefficients obtained are exhibited in Table 7. Major generalizations are:

TABLE 7

Measures of Relationship Pertaining to Upper Face Width, Lower Face Width, and Dental Arch Widths at the Deciduous Canines and Second Molars.

Variables Correlated	Males		Females	
	N	r	N	r
Upper c-c at age 4 : Upper c-c at age 8[1]	50	0.88	50	0.85
Lower c-c at age 4 : Lower c-c at age 8	50	0.80	50	0.84
Upper c-c at age 4 : Upper c-c gain age 4-8	50	0.09	50	—0.05
Lower c-c at age 4 : Lower c-c gain age 4-8	50	—0.02	50	—0.15
Lower c-c gain 4-8 : Upper c-c gain 4-8	50	0.51	50	0.59
Upper maximum c-c : Upper minimum c-c[2]	73	0.89	77	0.87
Lower maximum c-c : Lower minimum c-c	73	0.85	77	0.85
Upper maximum c-c : Lower maximum c-c	73	0.73	77	0.78
Upper maximum c-c : Upper maximum e-e[3]	73	0.80	77	0.77
Lower maximum c-c : Lower maximum e-e	73	0.79	77	0.69
Upper maximum c-c : Bizygomatic diameter	73	0.24	77	0.28
Lower maximum c-c : Bigonial diameter	73	0.10	35	0.25

[1] The symbol c-c in this and the four succeeding rows denotes maximum transverse distance between the deciduous canines.
[2] The r's in this row and the rows that follow are based on children 5 years of age.
[3] The symbol e-e denotes maximum transverse distance between the deciduous second molars.

1. The relationship between maximum bicanine diameter at age 4 years and maximum bicanine diameter at age 8 years, in white children subgrouped according to sex and dental arch, approximates $r = 0.85$. Meredith & Hopp ('56) obtained a somewhat higher association ($r = 0.90$) on comparable subgroups studied with reference to interbuccal width between the deciduous second molars.

2. No statistically significant relationship is found between maximum bicanine diameter at age 4 years and change in this dimension over

the succeeding four-year period. Coefficients near zero also were secured by Meredith & Hopp ('56) from size-increment correlations on corresponding age-arch-sex subgroups for interbuccal width at the deciduous second molars.

3. An r of 0.55 typifies the degree to which change in maxillary bicanine diameter over the period 4 to 8 years is associated with change in mandibular bicanine diameter over the same period. Meredith & Hopp ('56), in their study of dental arch width at the deciduous second molars, obtained concomitance of the order $r = 0.70$ for change in the upper and lower arches from 4 to 8 years. It follows that over the four-year age span under consideration there is a moderate positive relation between changes in width of the two arches at the deciduous second molars and an appreciably weaker positive relation between changes in width of the two arches at the canines.

4. The association between maximum bicanine diameter and minimum bicanine diameter, in white children 5 years of age grouped according to sex and dental arch, approximates $r = 0.86$. Meredith & Cox ('54), studying the relation between comparable dimensions taken at the permanent first molars on white children 9 years of age, secured an almost identical result.

5. Maximum bicanine diameter of the maxillary arch and maximum bicanine diameter of the mandibular arch, in white males and females 5 years of age, are related to the extent symbolized by $r = 0.75$. Similar coefficients have been reported for maximum width between the deciduous first molars in white children 2 to 5 years of age (Smyth & Young, '32), maximum width between the deciduous second molars in white children 4 years of age (Meredith & Hopp, '56), and maximum width between the permanent first molars in white children 9 years of age (Meredith & Cox, '54).

6. The intensity of association of maximum bicanine diameter and maximum width between the deciduous second molars is about the same as that of maximum bicanine diameter in the two arches. On specific sex-arch subgroups, all representing white children 5 years of age, the r's vary from 0.69 to 0.80. It will be recognized that these coefficients reflect individual differences in dental arch form or contour.

7. A low positive relation in the vicinity of $r = 0.25$ is indicated for bizygomatic diameter correlated wth maximum bicanine diameter of the maxillary arch. The specific r's on white males and females

5 years of age are each significant at the 5 per cent level of confidence. Coefficients of similar magnitude have been reported by Smyth & Young ('32) for bizygomatic diameter and maxillary arch width at the deciduous first molars ($r = 0.33$ on white children 2 to 5 years of age), and by Meredith & Higley ('51) and Meredith & Hopp ('56) for bizygomatic diameter and maxillary arch width at the deciduous second molars (r's $= 0.32$ and 0.37 on white children 5 and 6 years of age).

8. The coefficients derived by correlating bigonial diameter and maximum bicanine diameter of the mandibular arch are not statistically significant. Statistically significant associations for bigonial diameter and mandibular arch width at the deciduous second molars have been obtained at ages 5 and 6 years (Meredith & Higley, '51, Meredith & Hopp, '56). Close estimates for both relationships must await further research.

By considering together the materials colligated under 7 and 8 above, an integrative statement on the prediction of dental arch width from face width[2] was formulated. To wit: In the use of either bizygomatic diameter or bigonial diameter to predict dental arch diameters at the deciduous molars or canines, the index of prediction efficiency is less than 10 per cent.

Summary

Major sections of this paper have presented original findings on (1) mean bicanine diameter at selected postnatal ages between 4 and 8 years, (2) absolute and relative variability of bicanine diameter during childhood, (3) individual differences in growth of dental arch width at the canines over two biennia and one four-year period, and (4) associations between bicanine diameter on the two arches, size and growth of bicanine diameter, bicanine diameter and arch width at the deciduous second molars, bicanine diameter and face width.

The subjects are North American white children predominantly of northwest European ancestry and above average socioeconomic status. There is a main sample of 100 children studied at annual intervals from age 4 years to age 8 years, and an enlarged sample of 150 children studied at age 5 years.

Illustrative results are: (1) mean bicanine diameter is smaller at age 4 years than age 8 years by 2.6 mm, and smaller on girls than

[2] In this connection see Izard ('27) and Berger ('38).

boys by about 1.2 mm; (2) bicanine diameter is more variable in the maxillary arch than the mandibular arch, and more variable at ages 4 and 5 years than ages 7 and 8 years; (3) over the age interval 6 to 8 years, gain in bicanine diameter is less than 0.9 mm for 1 child in 10, between 1.4 mm and 2.4 mm for 1 child in 2, and more than 2.9 mm for 1 child in 10; and (4) there is a fairly strong positive relation between bicanine diameter at ages 4 to 8 years, a moderate positive relation between bicanine diameter in the two arches, a fairly weak positive relation between change from 4 to 8 years in the two arches, a weak positive relation between bicanine diameter and bizygomatic diameter, and no relation between bicanine diameter at age 4 years and change in bicanine diameter from 4 to 8 years.

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FACTOR ANALYSIS OF GROWTH OF CALIFORNIA MULE DEER ODOCOILEUS HEMIONUS CALIFORNICA

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INTRODUCTION

One of us (J. V. M.) has had the opportunity to take measurements for the last few years on deer killed by hunters near Fresno, California. More specifically the area lies just south of the confluence of the Middle Fork Kings River and the South Fork Kings River and includes portions of both Fresno and Tulare Counties. The area is officially defined as the Barton's Flat Deer Range. Funds were provided by the Pittman-Robertson Act, Federal Aid Program, Project PW41R California Big-Game Studies to make an intensive study of the deer in this area from the standpoint of maximum harvest and the results are being published elsewhere. Consistent with the general aims of the study various deer measurements were taken at the official checking station at Big Stump, Kings Canyon National Park. All measurements were on field dressed deer. Measurements were taken principally to determine (1) the general physical characteristics of the deer in this region, (2) effects of increased deer harvest on these characteristics, and (3) the relationships between size measurements of the deer for different age classes. The present paper is concerned with the third item.

Only male deer (California Mule Deer, *Odocoileus hemionus californica*) are considered in this study. Weight is of primary interest but other body and antler measurements were made in order to study the relationships of the measurements and the changes of these relationships with the age of the deer involved.

The essential methods of analysis are known in the literature as factor analysis as explained by Cattel (1952), Holzinger and Harman (1941) and Thurstone (1947). The conventional factor analysis has

been given a novel twist and applied in studies similar to the present one by Baker (1954).

Data

The data consist of measurements on deer killed by hunters from 1952 to 1954 inclusive. Most of the deer were killed during the regular hunting season but some were killed during special seasons. Time of killing was recorded but because of the small number in some categories the two times of killing have not been analyzed separately. The measurements and determinations made are as follows:

1. Age—the age of each animal was determined and recorded as yearling, 2-year old, etc.
2. Weight—the weight of the field dressed deer was taken to the nearest pound.
3. Girth—the girth was measured behind the shoulders around the heart region to the nearest ½ inch.
4. Total length—the length of the deer was measured from nose tip to base of the tail to the nearest ½ inch.
5. Height to the first fork—the height to the first antler fork or branch was measured on the left antler only and was taken from the base of the antler burr around the outside curve to the first crotch of the first fork to the nearest ¼ inch.
6. Total height of antler—the height of the left antler was measured from the base of the antler burr around the outside curve to the longest tine to the nearest ¼ inch.
7. Greatest width—the greatest span of the antlers was measured at the widest spread to the outside of each antler to the nearest ¼ inch.
8. Beam diameter—beam diameter was measured on the left antler one inch above the antler burr. The small diameter was measured with vernier calipers to the nearest 1/10 millimeter.
9. Points—numbers of points were recorded separately for right and left antlers but in our analysis we have used simply the total number.

There are noticeable differences in the yearling deer killed in the regular and special seasons. One difference is in the number of points. This difference is due to the fact that during the regular season only male deer with branched (2 points or more per antler) are legal. During the special season deer of either sex and any age may be taken

by hunters. Hence, there is a large proportion of spike (one point per antler) deer among the yearling deer killed in the special hunts.

A second difference is between the weights for the two seasons of each year for the yearling age class. Two things may be primarily responsible for the smaller deer. First, an order of dominance occurs whereby the dominant individual has first preference to food. Second, the smaller yearlings may be born at a later date. It is only fair, however, to include the kills of both seasons in order to provide a complete cross-section of the males of this species at this age.

Not all measurements are available on all deer. Some of the reasons for so much missing data are undetermined age, broken antlers, missing left antler, no branch on the left antler, girth split, deer cut in pieces, missing body parts, animal wrapped so as to be inaccessible, and rush periods when only partial data could be obtained.

We note that the environmental conditions contribute much to the animal characteristics of that year. The special hunts may change food supplies sufficiently to produce noticeable changes in the relationships of the measurements of the deer. The special hunts are held the last 12 to 15 days of November. The regular seasons are during September and October.

The data available are summarized in table 1. The means and standard deviations are given for all deer on which each measurement was made. The number of measurements in each case is indicated.

Analysis of the Data

Scatter diagrams were made for all the pairs of variables for each of the ages. The regressions seemed to be approximately linear. The correlation coefficients were estimated roughly. On this basis the variables height to first fork and greatest width were eliminated as not having any association with weight at any age.

For the other variables all deer were listed at each age for which all measurements were available. This cut the numbers somewhat severely. No distinction was made between deer in the regular and special seasons for the present purpose of determining the relationship between the measurements for different ages.

All deer in the age groups, yearlings, 2-year, 3-year, 4-year, 5-year and 6-8 year, that had values for each of the six variables (weight, girth, length, total height of antlers, beam diameter of antlers, and

total number of points) were included and correlation coefficients were calculated for all pairs of variables for each age group. These correlation coefficients gave rise to six 6 x 6 correlation matrices. A

TABLE 1

Means, $\bar{x}$, Standard Deviations, s, for Designated Numbers, n, for Eight Variables for Male Deer in Eight Age Groups.

Measurement		Age 1	2	3	4	5	6	7	8
Weight	$\bar{x}$	70.94	89.54	100.78	116.58	124.67	130.64	133.71	139.75
	s	10.72	11.11	16.24	17.02	17.01	15.40	13.27	23.19
	n	98	104	76	57	45	28	34	16
Girth	$\bar{x}$	31.09	34.09	35.64	37.60	38.05	39.14	38.55	39.04
	s	2.04	2.18	2.41	2.24	2.50	2.09	2.36	3.25
	n	74	76	57	46	37	22	28	12
Length	$\bar{x}$	49.68	53.35	55.05	56.03	57.23	57.62	58.03	57.59
	s	2.92	2.54	2.93	3.37	2.61	2.18	2.76	3.30
	n	98	103	75	57	41	24	32	16
Height to First fork	$\bar{x}$	6.05	6.84	7.20	7.76	8.30	8.19	8.88	9.78
	s	1.07	1.04	1.42	1.66	1.92	1.87	2.31	2.10
	n	77	131	101	77	52	32	35	17
Total Height	$\bar{x}$	8.13	12.01	13.85	16.16	17.99	18.18	19.54	21.01
	s	2.64	2.22	3.09	2.86	3.09	3.20	3.43	3.67
	n	110	140	104	78	52	32	35	17
Greatest Width	$\bar{x}$	7.51	11.67	13.49	15.93	17.52	19.11	19.15	21.81
	s	2.24	2.25	2.91	2.43	2.57	1.55	3.44	4.22
	n	68	89	56	41	30	21	26	13
Beam Diameter	$\bar{x}$	15.82	19.69	22.24	24.58	28.12	29.15	30.05	32.02
	s	2.78	2.42	3.25	3.74	3.56	2.86	3.56	4.65
	n	114	141	96	63	42	23	27	13
Points	$\bar{x}$	3.23	4.21	4.91	5.86	6.40	6.28	5.97	7.00
	s	0.86	0.76	1.37	1.50	1.47	1.28	1.37	3.25
	n	130	143	106	76	55	32	38	18

factor analysis was carried through to three factors in each case. Three factors were sufficient to reduce the elements of the residual matrices practically to zero in every case.

The 3 x 6 factor matrices were then rotated so that all of the determination of weight occurred in the first column. This was accom-

plished by using Lagrange multipliers and determining the F'_1 column of the λ-matrix (see Cattell (1951) p. 195) so that the weighting of the variable, weight, on the first factor, F'_1, is as large as possible. The weightings of the other variables on the first factor, F'_1, of the rotated factor matrix indicate the importance of these variables in connection with the deer-weight variable. The size of the deer-weight factor weight indicates its over-all connection with all of the other variables as a whole set of variables. The determinability of the deer-weight variable is measured by the square of its first factor weight and its uniqueness or independence of the other variables is meas-

TABLE 2

Male Deer Weight in Terms of a Profile on Five Variables for Six Age Groups—F'_1 of a Rotated 3 x 6 Factor Matrix.

	Age					
Measurement	1	2	3	4	5	6-8
Weight	.86	.87	.95	.88	.97	.96
Girth	.60	.64	.88	.78	.73	.72
Length	.48	.61	.46	.58	.21	.32
Total Height	.48	.34	.77	.73	.51	.21
Beam Diameter	.54	.43	.70	.71	.03	.37
Points	.41	.27	.23	.30	.21	.03
Number (n)	39	55	30	35	25	35
h^2 (%)	74	76	90	77	94	92

ured by 1 — (square of first factor weight). Thus, 74% of the variation in the weight of the yearling deer is due to something in common with the other five variables and 26% is due to something specific to the weight variable itself. This first factor, F'_1, of the rotated matrix could fittingly be called the deer-weight factor.

The details of the profiles of deer-weight factor for the additional five variables are given in table 2.

Discussion

The values of the means and standard deviations for the different age-groups given in table 1 indicate the growth curves and variabilities at different ages of the male deer. There are two factors that may cause distortion as compared to a growth curve determined from the same animals under the same conditions. First, these deer were born and lived during different periods of time which might imply quite

different environmental and competitive conditions. Second, there may be a pronounced selective mortality. This latter possibility is partially indicated by the erratic behavior of the figures for the 8-year old age class. Of course, the numbers involved in this class are very small but the unusualness of these figures seems to indicate something more than random deviations.

Table 2 essentially compares the weight-factors for the different age groups as expressed as profiles of five related variables. The size of the numbers indicates the importance of each of the variables from the standpoint of weight. For instance, all variables are important (have loadings above 0.40) for yearling deer. The over-all determinability of weight of yearling deer in terms of the other variables is 74%. This over-all determinability ranges up as high as 94% for 5 year olds.

The weightings for the different variables change strikingly with age. Girth loadings are high for all groups but are somewhat higher beginning with age 3 and after, being especially high for 3-year olds.

Length is of moderate importance until age 4 and after that has little effect on weight.

Total height of antlers is especially important for 3- and 4-year olds.

Beam diameter is of moderate importance for 1- and 2-year olds, of higher importance for 3- and 4-year olds, and of little importance thereafter.

Total number of points is of some importance for yearlings but of little importance thereafter.

An analysis of these data could have been made on the basis of multiple regression analysis with results that would have led to interpretations similar to those presented here with respect to the relative importance of the different variables for the various age groups. However, the analysis would not have been as straightforward and the comparisons would not have been as clear-cut.

As has been pointed out, the growth curves of the various measurements given in table 1 cannot be regarded as the growth curves of deer that are the same throughout a life span. However, these curves can be regarded as the curves actually observed under the conditions specified. With these facts in mind it may be noted that girth and length did not increase after age 6 years but that weight was still increasing at 8 years of age. The highest average weight

reached was 140 pounds, the greatest average girth attained was 39 inches, and the greatest average length was 58 inches.

The growth curves are the typical sinusoidal curves found by other investigators. Parts of the curves can be rather well approximated by straight lines. For instance, the weight curve can be fairly well represented by the equation

$$\text{weight} = 59.5 + 13.5\ (\text{age})$$

for ages 1 to 5 inclusive where weight is measured in pounds and age in years. This equation is not very refined but may be of value in field observations.

Summary

Partial data on three body measurements and five antler measurements have been presented for male deer (*Odocoileus hemionus californica*) for eight age groups for the following numbers of individuals:

Age group	*Number of individuals*
1	139
2	150
3	118
4	78
5	55
6	35
7	38
8	18

The means for the various age groups give a gross approximation to the growth curves for the deer but these curves may be distorted by changing environment and selective mortality. However, they can be regarded as the growth curves for the deer under the conditions that actually obtained.

The standard deviations of the various measurements show definite differences for the different age groups.

The extent to which the weight of the deer can be determined in terms of five other related variables varies from 74% for yearlings to 94% for 5-year olds.

The extent to which five related variables are affected by the

weight-factor varies markedly from one age group to another. All five are important for yearlings. For 2-year olds girth, length, and beam diameter of the left antler are important. For 3-year olds girth, length, total height of antlers, and beam diameter of antlers are important. For 4-year olds girth, length, total height of antlers, and beam diameter of antlers are important. For 5-year olds girth and total height of antlers are important. For 6-8-year olds only girth is important with minor emphasis on length and beam diameter of antlers.

Changes in environment including severity of competition may well account for many of the irregularities in tables 1 and 2. Some of the fluctuations are due simply to sampling since some of the numbers are rather small. However, the general picture seems quite clear and it is not expected that more extensive data of a similar nature would change markedly the striking and progressive change of the profiles of these variables with age as indicated in table 2.

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THE RELATION BETWEEN THE CONCENTRATION OF POTASSIUM CYANIDE AND GROWTH AND RESPIRATION IN *CHILOMONAS PARAMECIUM*[1]

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Since the work of Mast and Pace (1933) in which they reported success in axenic culture of *Chilomonas paramecium*, this organism has been used extensively for investigations in the field of cellular physiology. *Chilomonas* is a small (about 15 μ x 30 μ) colorless protozoan cell containing, under normal conditions, many starch granules and fat globules. The starch and fat content varies with certain changes in its environment or with age.

The rate of respiration in *Chilomonas* was investigated first by Mast, Pace and Mast (1936). The organisms were grown in an acetate-ammonium solution without addition of thiamine and iron. Using the direct method of Warburg they reported an oxygen consumption of 17 mm^3 per 10^6 organisms per hour for chilomonads from 72 hour cultures. Hutchens (1941) using a similar solution, obtained results indicating that oxygen consumption in *Chilomonas* varies inversely with the age of the culture. He later reported (1948) that young chilomonads (24 to 48 hour culture) utilized oxygen at the increased rate of about 50 mm^3 per 10^6 cell hours. Hutchens (1940) identified cytochrome c in *Chilomonas* and later found that cyanide (10^{-4} M) inhibits respiration.[2] In view of this fact and the rather unique metabolism of the species and since at the present time nothing has been reported on the effect of cyanide on growth in *Chilomonas*, the following investigations were made.

[1] The author wishes to express his appreciation to Donald M. Pace who suggested this problem and under whose guidance it was done.

[2] Hutchens (personal communication) has found that, "cyanide at about 10^{-7} M stimulates respiration 10-20% in *Chilomonas*. The inhibition curve centers on 10^{-4} M HCN and 80-90% of the respiration is cyanide-sensitive."

MATERIAL AND METHODS

The chilomonads used for this study were cultured in sterile acetate-ammonium (ac-am) solution (Pace and Ireland, 1945). The pH of the solution was adjusted to 6.8 with HCl. Pyrex glassware, reagent grade chemicals and triple distilled water were employed in all of the experiments. The organisms were grown under aseptic conditions in 125 ml. Erlenmeyer flasks each containing 70 ml. of solution and sealed with plugs of non-absorbent cotton wrapped in gauze. Inoculation was accomplished by the addition of 1.0 ml. of a flourishing 72 hour culture. The cultures were incubated in a constant temperature chamber at 24° ± 1° C.

The sterile cyanide stock solutions were prepared by adding aseptically 6.51 Gms. of KCN to a sterile 100 ml. volumetric flask. Sterile acetate-ammonium solution was added to volume. The concentration of the cyanide was strong enough to destroy any organisms introduced when the dry chemical was placed in the flask. To prepare a 10^{-2} M cyanide solution 1 ml. of the KCN stock solution was added to 99 ml. of sterile acetate-ammonium solution. All of the required concentrations of KCN were prepared in this manner.

The number of organisms in the cultures was ascertained every 24 hours and occasionally specimens were taken at random, stained with either Lugol's solution or Sudan III, and sketched with the aid of a camera lucida.

Rates of consumption of oxygen and production of carbon dioxide were measured by means of the "indirect method of Warburg" (Umbreit, Burris and Stauffer, 1949). In the statistical measurements the "t" test was used in evaluating the significance of the group differences.

RESULTS

1. *Effect of Cyanide on Growth*

Potassium cyanide was added to the culture solution in the following concentrations: 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, and 10^{-9} M. Ten 125 ml. pyrex Erlenmeyer flasks were used for each concentration and 70 ml. of solution were added to each. A similar number of flasks were prepared without cyanide to serve as controls. To each flask 1.0 ml. of solution from a flourishing 72 hour *Chilomonas* culture was added. A piece of sheet rubber was then placed over the cotton plug of each flask and held in place by a

rubber band. The cultures were maintained at a temperature of 24° ± 1° C. for 72 hours, at which time the organisms were counted. The results are presented in Table I. Preliminary tests had shown

TABLE I

Effect of KCN on Growth in *Chilomonas paramecium*

*, Significant Difference (P = < 5%); **, Highly Significant Difference (P = < 2%).

Molar conc. KCN	No. of tests	Av. no. cells per ml.	Test as % of control
0	4	690,000	2.2**
10^{-4}	10	15,200	
0	4	602,500	61.7**
10^{-5}	10	372,000	
0	4	655,000	89.3*
10^{-6}	10	585,000	
0	4	577,500	103.5
10^{-7}	10	598,000	
0	4	545,000	116.1*
10^{-8}	10	633,000	
0	3	590,000	101.0
10^{-9}	10	596,000	

that in 10^{-3} M KCN movement of the organisms was retarded within one hour and all cells were dead within three hours. Consequently this concentration was not studied further. As compared to the control cultures, a very great decrease in growth occurred in 10^{-4} M KCN, while there was an actual increase in numbers in the cultures containing 10^{-8} M KCN. The organisms in 10^{-7} M and 10^{-9} M KCN showed no significant differences in growth from the control cultures.

In another group of experiments the flasks were prepared as before and maintained at the same temperature. In this case, however, the number of organisms was ascertained by direct count of an aliquot portion which had been aseptically removed at 24, 48 and 72 hours. The results are shown in Table II. After 24 hours there was a significant inhibition in growth in 10^{-4} M and 10^{-5} M KCN. After 48 hours, all of the cultures containing cyanide in concentrations less than 10^{-5} M showed an increase in chilomonads over and above those found in the controls at that time. However, after 72 hours the results were somewhat different; the only cyanide solution in which the organisms

showed any significant growth above that shown by those in the control cultures was in 10^{-8} M KCN. These results (after 72 hours) are similar to those presented in Table I.

From time to time, camera lucida drawings were made of the organisms grown in various cyanide solutions as well as of cells from cultures containing no cyanide. Sketches of representative specimens from the control cultures and the 10^{-4} M and 10^{-8} M cyanide solutions

TABLE II

The Effect of KCN on Growth in *Chilomonas paramecium*. Ten Cultures Were Tested for Each Condition; Organisms Counted at 24, 48 and 72 Hours; *, Significant Difference (P = < 5%); **, Highly Significant Difference (P = < 2%).

Molar conc. KCN	Average No. of Organisms per ml. 24 hrs.	48 hrs.	72 hrs.
0	90,416	487,917	693,750
10^{-4}	603**	563**	749**
10^{-5}	43,667*	379,833	484,167**
10^{-6}	80,833	519,333	605,000*
10^{-7}	75,000	526,667	706,667
10^{-8}	90,000	590,000	847,500*
10^{-9}	102,500	499,167	695,000

are shown in figure 1. The animals grown without cyanide (control) had many starch granules and fat globules. The organisms exposed to 10^{-8} M KCN appeared to have slightly smaller quantities of starch and fat, but otherwise were similar to those in the control cultures. The most striking difference was observed in cells from the 10^{-4} M KCN where very little starch was evident, and the organisms were smaller and almost translucent. Fat globules were present in apparently normal amount.

2. *Effect of Cyanide on Respiration*

Solutions containing the same concentrations of KCN as those in the previous study were prepared and used for these experiments. Chilomonads from a 72 hour culture were used. The results are presented in Table III. All concentrations of KCN had an inhibiting effect on respiration, except in 10^{-9} M where oxygen consumption appeared to be slightly accelerated.

An average of the results of all of the control tests for oxygen consumption and carbon dioxide elimination (150 determinations) showed

the oxygen consumption to be 19 mm^3 per 10^6 organisms per hour. The carbon dioxide elimination was 19.5 mm^3 per 10^6 organisms per hour. The average R.Q. for the cells in all of the control tests is, therefore, just slightly above 1.0. This was also the average for organisms in most of the cyanide solutions. One outstanding exception was the R.Q. for those in 10^{-4} M KCN. The chilomonads in this solution grew and developed very poorly as shown in the figure.

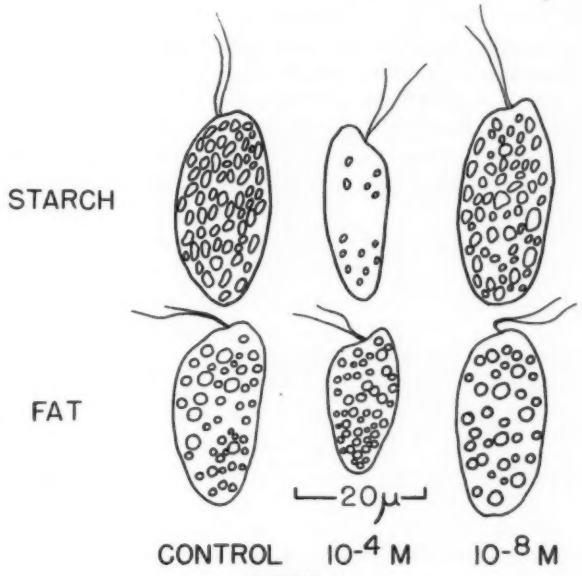

FIGURE 1

Camera lucida sketches illustrating starch and fat content, as well as size, of typical specimens obtained from control solutions as compared to those from solutions containing 10^{-4} M and 10^{-8} M KCN.

Discussion

From the results presented here it is evident that *Chilomonas paramecium* is cyanide-sensitive and its growth practically ceases in strong concentrations. A 99 per cent inhibition in 10^{-4} M KCN is similar to the inhibitory effect reported by A. Lwoff (1933) on several other flagellates. It is therefore quite probable that respiration in *Chilomonas* depends upon a cytochrome-cytochrome oxidase mechanism.

The normal oxygen consumption, 19 mm^3 per 10^6 chilomonads per hour reported in this paper, compares favorably with earlier measurements. Mast, Pace and Mast (1936) obtained an average oxygen consumption of 17 mm^3 per 10^6 organisms per hour for 72 hour cultures, while Hutchens (1941) reported a range of 11 to 17 mm^3 per 10^6 organisms per hour which varied inversely with the age of the culture. Mast, Pace and Mast (1936) found a R.Q. of 0.31 while

TABLE III

Effect of KCN on Respiration in *Chilomonas paramecium*. O_2 Consumption and CO_2 Elimination Expressed as mm^3 per 10^6 Cells Per Hour. **, Highly Significant Difference (P = < 2%).

Molar conc. KCN	No. of tests	Av. no. org./ml. (10^3)	Average O_2 consumption	Average CO_2 elimination	R.Q.
0	25	304	18	20	1.11
10^{-4}	27	304	10**	14	1.40
0	43	266	19	18	.94
10^{-5}	42	266	14**	15	1.07
0	21	268	17	17	1.00
10^{-6}	23	268	15	18	1.20
0	20	211	21	22	1.04
10^{-7}	18	211	19	20	1.05
0	23	340	20	21	1.05
10^{-8}	19	340	18	19	1.06
0	21	403	20	21	1.05
10^{-9}	20	403	22	24	1.09

Hutchens (1941) reported 0.75. The respiratory quotient from this study was very near to 1.0, which is the value expected from oxidation of either starch in the organisms or of acetate contained in the media. This difference might be explained by the fact that conditions to which the chilomonads were exposed differed in this work from the earlier investigations (thiamine and iron were included here) and also by the fact that in using the "indirect method of Warburg" carbon dioxide is not removed from the environment. It has been known for some time that carbon dioxide is necessary for the normal growth of *Chilomonas* (Mast and Pace, 1933; Jahn, 1936; Pace and Ireland, 1945).

In the higher concentrations of cyanide (10^{-4} M) a R.Q. of 1.4 was obtained and it was found that the organisms contain little starch, although the fat content remains about normal. It is known that if carbohydrate is being converted into fat within an organism, the R.Q. may reach a value of 1.4. Pace (1945) stated that one of the important factors in determining the degree of sensitivity of respiration in an organism to KCN is the concentration of carbohydrate in the cell. The results presented in this report are in agreement, since KCN definitely restricted the amount of carbohydrate in the cell.

A correlation between the results obtained for the growth and respiration tests is hardly feasible, since growth was observed at the end of 24 hours, whereas the respiration rate was observed for only 4 hours with organisms from a 72 hour culture. It has been shown, however, that in general those concentrations of cyanide which strongly inhibit respiration also inhibit growth.

It will be noted that in the growth studies the cultures averaged 600,000 to 700,000 cells per ml. while in the respiratory determinations the average number of organisms was about 300,000 cells per ml. The difference in the number of cells may be due to the higher carbon dioxide tension and the lower oxygen tension in the growth studies, resulting from the use of sealed flasks to prevent the escape of the cyanide. Pace and Ireland (1945) reported similar results from increased carbon dioxide concentrations.

The increase in cell numbers and the stimulation of respiration in low concentrations of KCN as well as other inhibitors has not been satisfactorily explained, although observed to hold true in other protozoans (Pace and McCashland, 1951; McCashland and Pace, 1952). Perhaps the cyanide activates a normally inactive system, or perhaps it depresses processes or mechanisms within the cell that function normally in inhibiting too great an activity.

Summary

1. Concentrations of KCN from 10^{-6} M and higher inhibit growth in *Chilomonas paramecium*.
2. In 10^{-8} M KCN a 16 to 22 per cent increase in cell numbers was observed as compared to the controls.
3. There is very little starch, if any, stored in the organisms exposed to 10^{-4} M KCN although the fat content is about the same as in the controls. The R.Q. in this solution is 1.4.

4. The oxygen consumption in the normal acetate-ammonium solution is 19 mm^3 per 10^6 organisms per hour, and the carbon dioxide elimination is 19.5 mm^3 per 10^6 organisms per hour. Normally, therefore, the R.Q. is approximately 1.0.
5. Oxygen consumption in *Chilomonas* is inhibited by concentrations of 10^{-8} M KCN and stronger but in 10^{-9} M KCN there is a 10 per cent increase in oxygen consumption.

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STUDIES OF THE NEW HAMPSHIRE CHICKEN EMBRYO

VII. Ultracentrifuge Patterns of the Plasma Lipoproteins

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Previous publications from this laboratory have described certain density fractions of plasma lipoproteins, as observed during embryonic development, in terms of nitrogen and total lipide (1) (2) and also with respect to proportions of triglycerides, sterol esters, non-esterified sterols, and phospholipides (2) (3). To supplement these findings, and to more intimately relate them to the various molecular species, it is desirable to obtain photographic records of the components as resolved by centrifugation in the analytical ultracentrifuge.

In this paper are presented the schlieren patterns observed during ultracentrifugation of lipoproteins obtained from the plasmas of chicken embryos of various developmental stages.

Also, since this paper marks the termination of a phase of study describing origins, concentrations, and physico-chemical properties of various lipoproteins in the plasmas of normal chicken embryos, this and previously obtained data are examined with reference to growth curves of the whole embryo and the liver.

Materials and Methods

For each experiment with embryos, blood was pooled from at least 24 birds. In the case of newly-hatched chicks, 4-day old chicks, 14-day old chicks, and 28-day old chicks, blood was usually pooled from 12 to 15 birds. Three roosters provided the blood for studies on the adult.

Separation procedures were carried out prior to ultracentrifugal

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analysis. These included (a) isolation of lipoproteins which floated on solutions of densities between 1.003 and 1.063 and, (b) separation of lipoproteins which floated on solutions of density 1.21. These preliminary lipoprotein fractionations were performed with partition cells devised in this laboratory (4) and by techniques described in previous publications (1) (3).

Analyses of the lighter (beta?) lipoproteins were carried out at 39,460 r.p.m. (solvent density 1.063, NaCl) in the Spinco ultracentrifuge (Model E)*. Analyses of denser lipoproteins were performed at 50,740 r.p.m. (solvent density 1.21, KBr).

Measurements of the movements of the boundaries were made with a microcomparator calibrated to one one-hundredth of a millimeter. From these data the floatation constants were calculated according to the procedures of Svedberg (5), de Lalla and Gofman (6) and Green, Lewis, and Page (7) and extrapolated to infinite dilution. Floatation rates of lipoproteins in solutions of density 1.063 are expressed in S_f units in accordance with the terminology of the Gofman group. Floatation rates of lipoproteins in solutions of density 1.21 are expressed in $-S_{1.21}$ units following the example of the Page group. Determinations of the concentrations of components floated in a sample were approximated as follows: floatation patterns were traced directly from the photographic plate to graph paper ruled in square mm (Figs. 1, 2, 3)**. A talley of the number of square mm occupied by a component gave a measure of its relative concentration. At this point Johnston-Ogston corrections are ordinarily applied. However, it was observed that this correction is essentially cancelled by a correction, usually ignored, which is based on the relative amounts of protein and lipide in a component. (Since the refractions of light of lipide and protein are in opposite directions relative to the solutions employed for these studies, a lipoprotein containing more nearly equal proportions of these components will net a lesser deflection than will one which contains proportionately more of one component). Uncorrected areas were therefore averaged and then converted to approximate milligrams per cent on the basis of previous nitrogen and lipide determinations on isolated fractions (1) (2) (3).

* This relatively low speed of centrifugation was chosen to permit a more precise measurement of floatation with minimal sacrifice of resolution. Also, several runs were made at solvent densities of 1.07, but appropriate corrections were made so that the resulting rates of floatation could be expressed relative to a density of 1.063.

** Since it seemed more convenient to compare the various deflections, if these were depicted as positive deflections, the bar was reversed so as to achieve this.

RESULTS

Concentrations of the various lipoprotein components changed considerably during embryonic development. As shown in Figs. 1, 2, 3, and 4, the major lipoproteins of embryos of 8 and 10 days incubation were those of medium density which floated at approximately S_f 8.* Lipoproteins having approximate mean floatation rates of S_f 3.5 and –S 7 were virtually absent from the plasma at these stages,

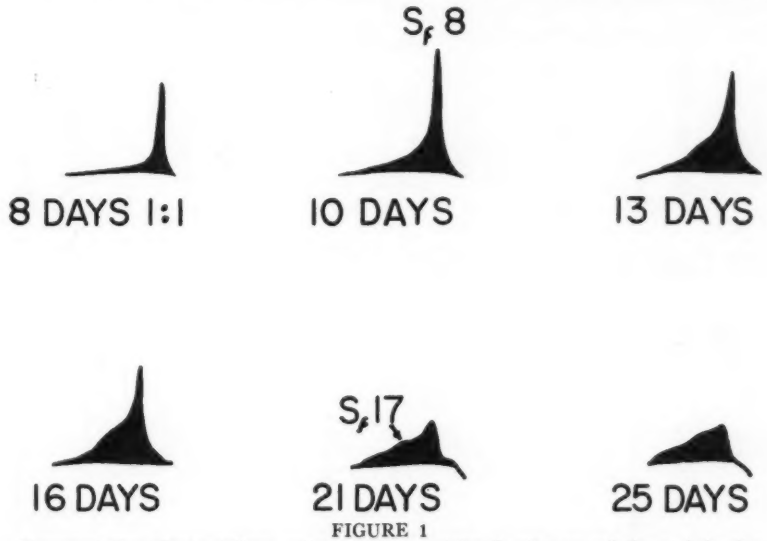

FIGURE 1

Representative ultracentrifuge patterns of lipoproteins floating on solutions of density 1.063 at different stages of development. Materials floating at density 1.003 removed by prior centrifugation. Photographs taken approximately 80 minutes after reaching speed 39460 r.p.m.

and denser (–S 4.5) and lighter (S_f 17) lipoproteins were present in low concentration. As development (including the growth of the liver) proceeded, the lighter S_f 17 complexes showed an increase (to the 18th day of incubation). This was apparently at the expense of the S_f 8 complexes (Figs. 1 and 4, and Table II), which species after 10 days, decreased progressively. At hatching, a small amount of com-

* These lipoproteins are the major proteins of the plasma at this stage, albumin being present in low concentration (Fig. 4) and non-lipoprotein globulins being virtually absent. It is not clear whether or not this low concentration of non-lipoproteins is related to the relatively high lipid content of the plasma.

ponent S_f 3.5 was first observed in the plasma (Fig. 2). The dense –S 4.5 lipoproteins increased relatively slowly from the 10th day of incubation but almost tripled in concentration close to the time of hatching. At the 16th day, the –S 7 component could sometimes be clearly resolved and some of this material was possibly present in the plasmas of younger embryos. The concentration of this species was increased approximately eight times between 18 days and hatching (Figs. 3 and 4).

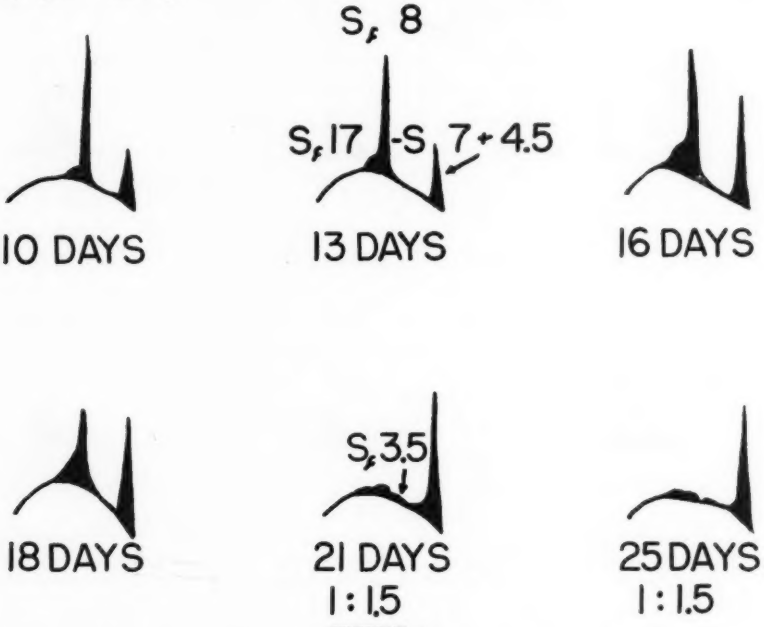

FIGURE 2

Representative ultracentrifuge patterns of lipoproteins floating on solutions of density 1.21 (KBr) at various stages of development. Photographs taken 17 minutes after start of run. Speed: 50740 r.p.m.

Corrected floatation rates and approximate milligrams per cent of the various components at each stage investigated are presented in Tables I and II. Percentages of protein and lipides in the lipoprotein species are listed in Table III. In Fig. 4 the concentrations of various lipoproteins and albumin, at different stages, are compared with the increase in weight of the whole embryo and the liver. Fig. 5

shows the result of centrifuging equal mixtures of lipoproteins from birds of different ages; and the schlieren patterns given by yolk lipoproteins floating in a solution of density 1.063 are presented in Fig. 6.

Discussion

In concluding this phase of study it would be desirable to state something definitive regarding lipoproteins and the processes which are associated with growth and differentiation. A number of correlations *do* exist between concentrations and types of lipoproteins and

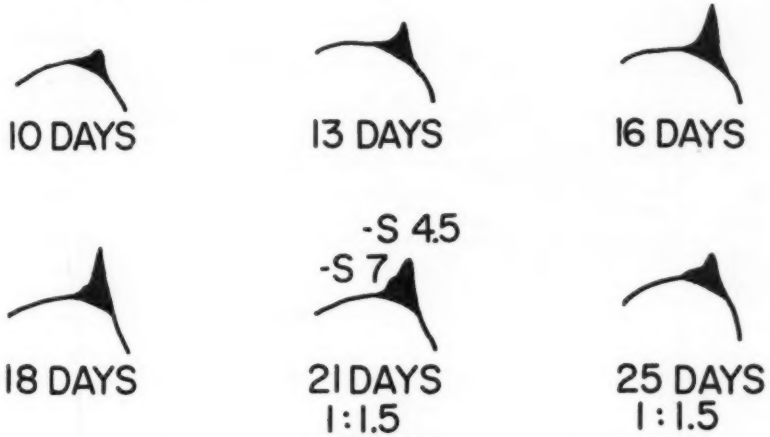

FIGURE 3

Representative ultracentrifuge patterns of –S 7 and 4.5 components at different stages of development. Ultracentrifugal medium: KBr, density 1.21. Photographs taken approximately 87 minutes after reaching speed 50740 r.p.m.

the development of the embryo as a whole and in part (Fig. 4). Unfortunately, these fall short of established relationships, and the main justification for their mention here is to provide suggestions for definitive experimentation.

"Chylomicrons" and Growth: Although no analytical centrifuge data was obtained for species floating on a solution of density 1.003, chemical measurements ("Chylomicrons", Fig. 4) indicate that their concentration increases slowly during incubation but falls off abruptly at hatching. Also, this fraction, at hatching assumes a radically different composition, consisting predominantly of sterol ester rather than

triglyceride. Table III indicates that very little replacement of triglyceride with sterol ester in this fraction has taken place prior to hatching. These facts point to either a relative reduction in supply of phospholipid and triglyceride from the yolk close to hatching or an increasing capacity of the organism to convert such materials. For

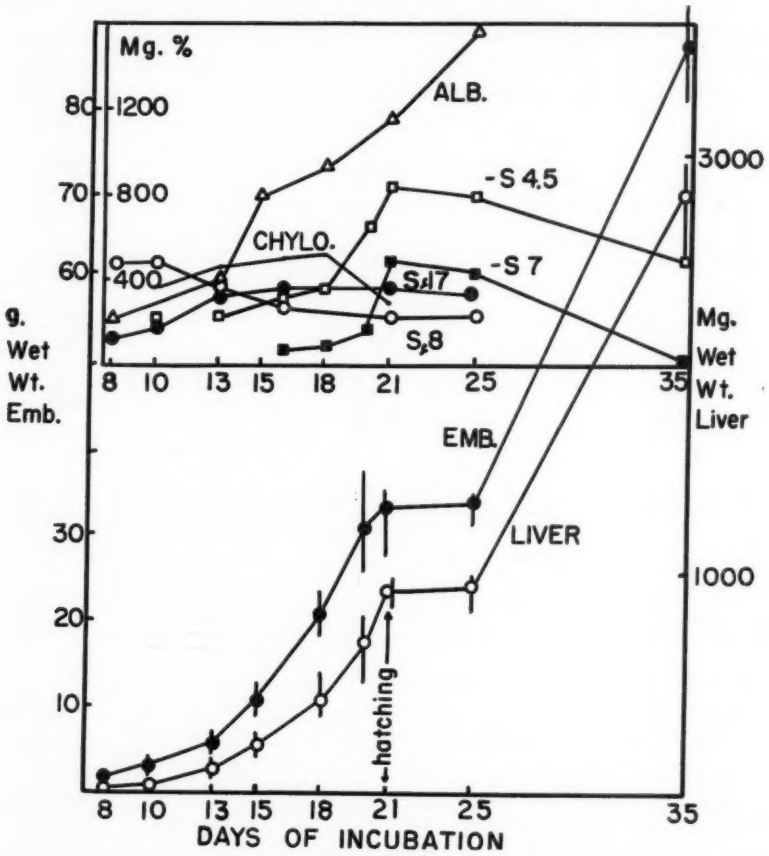

FIGURE 4

Comparisons of lipoprotein concentrations with growth of whole embryo and liver. At least 5 animals (usually 6 to 12) are represented by each point for whole body and liver. Vertical lines indicate the range. Protein values derived from chemical analyses of ultracentrifugal fractions (1) (2) (3) and Table II.

example, the liver may become more active in the conversion of triglycerides to sterol esters (and phospholipides)*. Entenman Lorenz, and Chaikoff (8) have found the liver of the newly hatched chick to contain unusually large amounts of sterol esters. The role of such

TABLE I

Mean Floatation Rates of Major Lipoprotein Components.

Age	1 (S_f)	2 (S_f)	3 (S_f)	4 (–S)	5 (–S)
Embryos					
8 days	17.0	7.3	?	?	?
10 days	17.6 (17.5-17.8)[3]	7.7 (7.6-8.0)[4]	Not Detected	Not Detected	5.0 (4.9-5.1)[2]
13 days	17.0 (16.3-17.6)[2]	8.4 (7.8-8.6)[2]	↓	↓	5.2 (5.0-5.4)[3]
16 days	16.6 (15.4-17.2)[4]	7.8 (7.7-7.9)[4]	↓	7.0	4.7
18 days	16.0	7.2	↓	7.6	4.4 (4.2-4.6)[2]
21 days	17.0 (16.0-19.0)[7]	8.0 (6.8-9.0)[6]	3.8 (2.8-4.6)[4]	6.9 (6.7-7.0)[3]	4.4 (3.9-4.9)[9]
Chicks					
4 days Fasted	16.0	7.7 (7.1-8.3)[2]		7.0	4.6 (3.4-5.5)[4]
14 days	?	?	?	Not Detected	4.6
28 days	?	?	?	↓	4.6
14 weeks	Not Detected	7.6	Not Detected	↓	4.7 (4.6-4.7)[2]

Floatation rates corrected to 20.0°C and extrapolated to zero concentration. Numbers at upper right of brackets enclosing ranges indicate the number of samples centrifuged. Where no range is indicated only one experiment was performed.

large amounts of sterol esters in the plasma and in such an unusual complex at this point in development remains obscure. Quite possibly this situation reflects inability of the chicken embryo to completely utilize the large amount of triglyceride available. Excess fatty acids, rather than being used for energy or synthesis

* Counts of Kupffer's cells of the liver (which may participate in the engulfment and breakdown of chylomicrons) show that these are markedly increased at about the time of hatching.

are shunted off (with the aid of increased liver synthesis of cholesterol) into this type of complex. When the chick reaches two weeks of age (on a lower fat diet and growing rapidly) these lipoproteins have largely disappeared from the plasma.

TABLE II

Estimates of Lipoprotein Concentrations (mg %) at Various Stages of Development.

Age	S_f 17	S_f 8	S_f 3.5	–S 7	–S 4.5
Embryos					
8 days	110	450	Not Detected	?	?
10 days	170 (170)[3]	470 (470)[3]	↓	Not Detected	200 (180-220)[2]*
13 days	275 (275)[3]	375 (375)[3]	↓	↓	240 (240)[3]*
16 days	335 (320-360)[4]	330 (310-360)[4]	↓	75	335
18 days	475	335	↓	50 (0-100)[2]	410 (360-450)[2]
21 days	380 (355-410)[5]	240 (200-280)[5]	40 (25-50)[5]	410 (330-510)[5]	1030 (930-1100)[5]
Chicks					
4 days Fasted	355 (355)[2]	245 (245)[2]		260 (260)[2]	970 (970)[2]
14 days	?	?	?	Not Detected	505
28 days	?	?	?	↓	460
Rooster					
14 weeks	Not Detected	120	Not Detected	↓	1000 (1000)[2]

All values of S_f 17, S_f 8, and S_f 3.5 components based on reference to previous lipide and nitrogen determinations on total 1.063 density fraction (1)(2)(3). Values for –S 7 and –S 4.5 in the 21 day embryo also based directly on previous lipide and nitrogen determinations (1), values for these components at other stages derived by reference to the 21 day stage. Ranges of relative refractions appear in brackets. Numbers at upper right indicate number of samples measured.

* These values (based on the amount of refraction of this component in the schlieren lens system) appear to be low according to lipide and nitrogen measurements of the dense protein fractions. The explanation may be that other lipoproteins are present over a broad range of densities and that these are not apparent as obvious refractions.

Medium Density Lipoproteins and Growth: According to curves in Fig. 4, the S_f 17 lipoproteins increase with increasing size of the whole embryo and the liver, whereas the S_f 8 species decrease almost proportionately. Indeed, if the concentrations of these two lipoproteins are

added together, their sum is fairly consistent throughout the embryonic stages studied, showing only a slight rise at 18 days of incubation (Table II). It seems possible that the increase in S_f 17 component is a reflection of the total synthesis of cholesterol esters by the liver, which is increasing in size during incubation. Chemical analyses (Table III), as well as the complimentary changes in proportions of S_f 17 vs S_f 8 molecules with development strongly suggest that the S_f 8 molecules are being converted to S_f 17 complexes mainly by the addition of sterol esters. (Increasing amounts of sterol ester in the plasma may also be responsible for the appearance, close to hatching of the S_f 3.5 and –S 7 species; the –S 7 complexes may represent –S 4.5 species to which sterol esters have been added.)

Otherwise it is diffcult to relate these two groups to the growth processes except to point out that their individual and combined concentrations are considerably higher than those of adult (non-growing) chickens (Table II) and most other species. Although such a pattern might be expected on the basis of the high proportion of fat in the embryo diet, it may be that these forms are also less effectively converted to denser forms (directly or indirectly) by the embryo.

Denser Lipoproteins and Growth:

Initial increases in the concentration of –S 4.5 and –S 7 components are small compared to striking changes taking place between 18 days of incubation and the time of hatching. The curves in Fig. 4 indicate that the increase in –S 7 component, at least, is greatest as the growth of the embryo and its liver are slowed. This is in contrast to the behavior of other proteins such as albumin, whose concentration increases progressively with age (Fig. 4) and seems uninfluenced by the cessation of growth at the time of hatching. A notion resulting from these findings is that the denser lipoproteins may be utilized during growth at rates which cause the concentration in the plasma to be very low. When growth ceases (temporarily in the case of the newly hatched chick) the concentration in the plasma is increased. When the chick is again actively growing (i.e., 2 weeks, 4 weeks, and 7 weeks post-hatching) the denser lipoproteins are once more reduced in the plasma. Since the lighter lipoproteins fail to show this correlation to the growth rate, it seems possible that a major portion of lipide and protein energy for embryos is conveyed to the cells in the form of denser lipoproteins.

TABLE III

PERCENTAGES OF PROTEINS AND LIPIDES IN VARIOUS DENSITY FRACTIONS

Age	Density 1.003					Density 1.003-1.03					Density 1.03-1.063				
	Prot.	S.E.	Tri.	S	P.L.	Prot.	S.E.	Tri.	S	P.L.	Prot.	S.E.	Tri.	S	P.L.
Embryos															
10 days	10.6	9.0	46.4	5.4	28.6										
13 days	12.2	10.9	43.6	5.6	27.7										
15 days															
16 days	11.5	11.0	45.8	5.7	26.0	17.5	39.7	6.1	9.2	27.5	22.4	29.6	8.9	6.0	33.0
18 days															
21 days	16.2	46.7	8.5	4.4	23.2	17.7	45.8	3.0	6.1	27.4	24.9	29.4	5.4	8.1	32.2
Chicks															
4 days															
Fasted															
Rooster															
14 weeks															
Adult															
Chicken															
Hillyard et al. (13)															
Adult															
Human															
Hillyard et al. (13)															
Adult															
Rabbit															
Hillyard et al. (13)															

Except for adult rooster, these values on chicken lipoproteins were largely derived from tables presented in previous publications (1)(2)(3). Nitrogen determinations were by the microKjehldahl technique. Total lipide was extracted by the Delsal (20) procedure. Lipide classes were determined by the chromatographic column technique of Fillerup and Mead (21). Corrections were applied for non-protein nitrogen including phospholipid nitrogen.

TABLE III (*Continued*)

PERCENTAGES OF PROTEINS AND LIPIDES IN VARIOUS DENSITY FRACTIONS

Age	Density 1.003-1.063					Density 1.063-1.21				
	Prot.	S.E.	Tri.	S	P.L.	Prot.	S.E.	Tri.	S	P.L.
Embryos										
10 days	20.7	24.1	12.0	5.8	38.0					
13 days	18.0	27.9	12.6	6.0	35.5	44.7	15.2	4.8	4.0	31.3
15 days						43.7	11.8	6.2	3.7	34.6
16 days	20.0	34.7	7.5	7.5	30.3					
18 days						43.3	19.4	2.5	3.8	31.0
21 days	21.3	37.1	4.2	7.2	29.8	45.0	19.8	5.0	2.6	27.6
Chicks										
4 days			*							
Fasted	21.4	43.5	2.6	6.2	26.1	45.0				
Rooster										
14 weeks						50.0	14.8	2.3	1.8	30.1†
Adult										
Chicken			*							
Hillyard et al. (13)	17	20	35	7	21	47.0	12.0	10.0	4.0	27.0
Adult										
Human			*							
Hillyard et al. (13)	17	29	25	8	21	55.0	13.0	6.0	2.0	24.0
Adult										
Rabbit			*							
Hillyard et al. (13)	17	19	37	6	21	59.0	10.0	6.0	3.0	22.0

Except for adult rooster, these values on chicken lipoproteins were largely derived from tables presented in previous publications (1)(2)(3). Nitrogen determinations were by the microKjehldahl technique. Total lipide was extracted by the Delsal (20) procedure. Lipide classes were determined by the chromatographic column technique of Fillerup and Mead (21). Corrections were applied for non-protein nitrogen including phospholipid nitrogen.

* All material floating at 1.063—correction not applied for amount of chylomicron material present.

† Results of only one experiment.

There is some evidence that in the adult the denser (alpha?) lipoproteins do not contribute directly to the nutrition of cells, except, perhaps, as transporting agents for non-esterified fatty acids. Fisher, et al. (9) have shown that the phospholipides in the adult rat are both synthesized and destroyed to a major extent in the liver. Gordon (10) has recently claimed that most of the lipid directly utilized

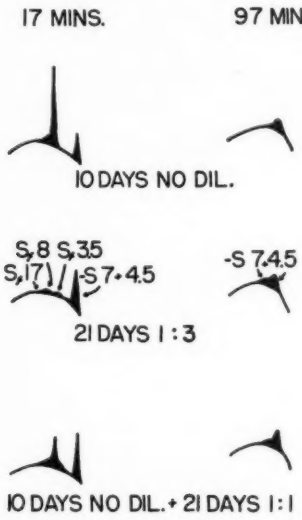

FIGURE 5

Ultracentrifuge patterns illustrating close similarity in floatation rates of major lipoprotein components present in plasmas of both 10-day old embryos and newly hatched chicks.

by cells in adult man is transported as non-esterified fatty acid in loose combination with albumin. However, whereas the adult circulation contains sufficient albumin to carry out such a function, the albumin in the early embryo (8 to 10 days incubation) is present in less than one-tenth of the concentration observed in the adult chicken. Perhaps the finding that frog eggs and larval cells contain and utilize large amounts of lipoproteins rich in phospholipide (11) also lends some support to the notion of preferential utilization of dense lipoproteins by growing cells.

Alternatives to the above interpretation are (A) that as growth is

slowed, the liver is automatically activated in the direction of synthesizing more alpha lipoprotein and that this plasma pool serves as a reserve which can be readily converted to usable fatty acid, amino acid, etc., by the liver upon demand, (B) that a specialized (hormonal?) stimulus is involved or (C) that it is not until this point in development that the liver is capable of this degree of synthesis.

FIGURE 6

Yolk lipoprotein floating in a solution of density 1.063 (8-day old embryo). Speed: 39460 r.p.m.

Uniformity of Lipoproteins: It appears that the major lipoprotein components observed during incubation are very similar in general composition (not necessarily concentration) from individual to individual and from stage to stage. For example, it is observed that resolution almost as fine as that seen in the plasma from individual adults is obtained in the pooled plasmas from 24 (and more) embryos. Also, within a reasonable margin of error, the mean floatation rates of these components seem identical at different stages (Table I). When lipoproteins from the plasmas of embryos incubated 10 days were mixed with lipoproteins from newly hatched chicks and centrifuged together, the resulting peaks gave no evidence of floatation differences according to embryo age (Fig. 5).

Chemical studies of these components (Table III) tend to support the notion that lipoproteins observed in normal plasma may exist as structural entities of rather closely defined lipide-lipide- and lipide-protein combinations*. Allowing a margin for experimental error, it

* In an earlier study it was erroneously reported that the –S 4.5 and 7 lipoproteins of younger embryos (10 to 18 days incubation) contained higher proportions of lipide than did those of hatching and post-hatching stages. Corrected data on these proteins has been incorporated into Table III.

can be demonstrated that lipoproteins floating at similar rates contain similar (not necessarily identical) proportions of protein and total lipide; and that the lipide moieties consist of similar proportions of the four major lipid classes (sterol ester, triglyceride, non-esterified sterol, and phospholipide).

Several hypotheses may be advanced to explain the persistence of specific lipide-protein and lipide-lipide combinations in the plasmas of developing embryos: (A) These particular combinations may be least susceptible to utilization. (B) Different classes of lipoproteins may exist as a result of definite and differing syntheses or modifications in the liver and other organs. It has also been suggested (C) that some of the lipoproteins of the plasma in embryos are incorporated whole from the yolk. From Fig. 6, it is clear that if this is the case, considerable modification must take place during the transfer from yolk sac to plasma.

Comparison with Lipoproteins in Plasma from Adults: The combination ultracentrifugal and biochemical studies of Lewis, Green, and Page (12), and Hillyard, Entenman, Feinberg, and Chaikoff (13) on lipoproteins of a variety of adult animals reveal some lipoprotein differences attributable to species, notably differences in proportions of protein to lipid (Table III). But where the groups correspond in density, for any one of these species, the proportions of lipide to protein and lipide to lipide seem by cursory inspection to be similar in composition to those of the chicken embryo (Table III). To ascertain whether major differences in rate of floatation exist between corresponding components of the plasmas of embryos and adult chickens, equal amounts of lipoproteins from 15-day embryos and 14-week old roosters were mixed and centrifuged together at a density of 1.21. As in the case of the 10-day old embryo and the newly hatched chick, no differences could be detected.

Concerning lipoprotein distributions, the S_f 17 species present in such high concentrations in the older chicken embryos are not detected in the adult rooster and are likewise absent in normal adult rabbits, rats, cats and dogs (12). Similar material is, however, present in the hen, and in humans, monkeys, guinea pigs and opposums. Since lipoproteins of this density class have been identified with coronary disease in the human being, it is not surprising that the chick is quite susceptible to experimental arteriosclerosis by cholesterol feeding.

Also, the so-called "chylomicrons" of the embryo are quite different from those normally seen in the adult. They contain considerably more phospholipid and protein and seem to be smaller than the majority of such complexes floating at this density (1.003) in the plasma of the adult hen. It cannot be definitely stated whether they are derivatives of chylomicrons or of lipoproteins.

Comparison with Electrophoretic Studies: Results of electrophoretic studies on lipoprotein changes occurring in the plasmas of embryos are confusing even when the buffer solutions employed by different investigators are taken into account. Thus, Marshall and Deutsch (14) and Heim and Schectman (15) using veranol and borate buffers respectively on chicken embryo serum, found an increase in so-called "beta" lipoproteins at hatching; whereas Moore, Shen, and Alexander (16) using phosphate buffer, demonstrated increases in lipoproteins migrating in the "alpha" region at this stage. (The "alpha" lipoproteins in the latter case may consist, in major part, of species floating at –S 3 to –S 7, since Lewis, Green, and Page (12), and Goldwater and Entenman (17) have found such correspondence in the lipoproteins of adults of various species.) Moore et al. (16) have demonstrated, in addition, that the alpha species is relatively high in the early pig fetus as compared to the chicken embryo of similar stage. The component decreases in the pig fetus at birth as contrasted to the sharp elevation seen in the hatching chicken. Assuming that the "alpha" components in the two species are the same, the large amounts of these materials present in early stages of the pig may be due to relative non-utilization. Several sources of energy may be forthcoming to the fetus via the placenta, (notably carbohydrate and fatty acids) and may be used preferentially. Alternative explanations are (a) that the pig liver has better developed systems for the production of this protein or (b) that the pig liver is not taxed to the same extent by the other types of conversions or syntheses.

Several reports have also described pre-albumin components (species migrating in advance of albumin) in the sera of chicken embryos and laying hens (14, 15, 18). Such species migrate fastest in sera from early embryos. Vanstone, et al. (18) demonstrated that, in the case of paper electrophoretic patterns, these materials take both carbohydrate and lipide stains. Centrifugation gives no direct evidence of such materials, but in younger embryos it was noted that the species observed to float as a discrete component at a rate of –S 4.5 (Table II)

did not seem to account for the total nitrogen and lipide detected chemically in this fraction. Whether or not the fast moving components are actually qualitatively distinct proteins is not clear, and is perhaps a matter of definition. The gradual slowing of migration rate of pre-albumin species with development (until they finally are no longer "pre-albumin" but are in the alpha lipoprotein region) suggests that they may consist of complexes between agents prevailing in the serum of the embryo at the time (carbohydrates and lipids) and a basic type lipoprotein or glycoprotein. Recent studies in our laboratory show that seromucoid type carbohydrate is indeed present in the alpha lipoproteins. Even results of immunochemical studies revealing antigens specific to the sera of embryos (19) do not necessarily imply the existence of qualitatively distinct proteins, since such specificity may be largely determined by haptens and/or masking.

Summary

Ultracentrifuge patterns were obtained of the lipoproteins in the plasmas of embryos in various stages of development. These are compared to growth curves of the whole embryo and the liver and to patterns obtained from growing chicks and adult roosters.

The sum of the lipoproteins of median density (approximately S_f 3-25) remained fairly constant throughout development, but a shift toward lighter from the denser of such components took place as the embryo increased its synthesis of sterol ester. The S_f 3.5 group was first detectable at hatching.

The dense lipoproteins (approximately –S 3-10) were present in relatively low concentration until just prior to hatching. Between 18 days of incubation and 21 days, several-fold increases were observed, which seemed to be correlated with the abrupt slowing of growth at this time. A shift from denser toward lighter components occurred after the 16th day of incubation.

Chemical determinations on isolated lipoprotein groups reveal that definite ratios of protein to lipide and lipide to lipide prevail for each density group in all stages studied.

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SOME OBSERVATIONS ON THE PITUITARY GLAND FOLLOWING THE PROLONGED ADMINISTRATION OF AN ANTITHYROID DRUG*

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During the past few years improvements in staining procedures have led to changes in our understanding of basic cellular morphology and physiology of the anterior pituitary gland. Two valuable staining procedures are available for the study of cell types in the rat anterior pituitary. They are the McManus periodic acid-Schiff (PAS) technique as first applied to the pituitary by Catchpole (1949), and Gomori's aldehyde-fuchsin stain as employed by Halmi (1950, 1952) and others. With the use of the above techniques, Purves and Griesbach (1951a and 1951b) have identified two types of basophils, the thyrotrophs and the gonadotrophs, chiefly on the basis of responses to hormone treatments. These investigators, as well as others, conclude that the basophils differ not only in their morphology and distribution in the adult rat pituitary but also in their physiology.

The experiments to be described in this paper were undertaken with an attempt to record the changes which might be encountered in the basophils of the rat anterior pituitary, especially the thyrotrophs, following the prolonged administration of thiouracil to a group of male rats. A preliminary report of this work has appeared (Clausen 1955).

MATERIAL AND METHODS

One hundred Sprague Dawley male rats, with an average weight of 60 grams, were divided into two groups. One group of 80 animals received a 0.2 per cent thiouracil solution as a substitute for

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the drinking water. The other group of 20 animals was carried along as the normal littermate control group. All of the rats were maintained at a fairly constant temperature and were given a normal stock diet of Purina dog chow which was supplemented with lettuce at intervals of several days. The animals were weighed each month, and the entire series in both groups was sacrificed at the end of 24 months by exsanguination under ether anesthesia. Fifteen animals in the experimental group and five animals in the control group were sacrificed 6, 14, and 18 months after the initiation of the experiment.

The animals were examined for gross pathological changes, and the following organs and tissues were removed and fixed in formalin, Bouin's or Zenker's fixative and embedded in paraffin for histological examination: thyroid, pituitary, pineal, adrenals, tongue, gingival tissue, teeth, heart, aorta, lung, prostate, testes, tibia, and skin. The pituitary glands, the glands with which this report is chiefly concerned, were cut serially in the horizontal plane at 5 μ. Every fifth section was stained by Gomori's aldehyde-fuchsin stain (Gomori, '50). Other sections were stained by Crossman's modification of Mallory's triple stain or with hematoxylin and eosin. Representative sections of many of the glands were also stained by the periodic acid-Schiff technique after the manner of Purvis and Griesbach ('51a). It was observed that the staining ability of various batches of the aldehyde-fuchsin stain would vary considerably. Some mixtures failed to stain the basophils several days after preparation, while other seemed to remain effective for several months. Therefore, the stains were always tested on control glands known to contain Gomori-positive basophils at the time it was used on the experimental pituitary glands.

Observations and Results

Sections of anterior pituitaries, after six months of thiouracil administration (Figure 2), showed evidence upon histological examination of a more diffuse distribution of the thyrotrophic cells and a hyperplasia of this beta cell type as compared to the similar basophilic cell type of the littermate control animals (Figure 1). The thyrotrophs appeared to be scattered thinly throughout the gland in the experimental animals, whereas in the control animals these cells showed an apparent tendency to aggregate in the more centralized

portion of the gland with a few scattered cells near the intermediate lobe and the periphery of the gland. Very little hypertrophy of the various cellular components was evident even though the glands had increased in weight approximately 10 per cent over that of the littermate controls.

A more pronounced change in the size of the pituitaries was evident at the end of one year of thiouracil administration. The weights of the glands increased from 20 to 25 per cent during this period. Histologically, there was an apparent cellular hyperplasia and hypertrophy with some increased vascularization similar to that which is always found in the thyroid glands of animals subjected to antithyroid drugs for 12 months. The histological picture of the 1 year old controls did not differ from those of 6 months. It was evident (Figure 3) that the thyrotrophs were, for the most part, more densely stained and the granules were more compact in most of the basophils at this stage. There was a scattering of cells which showed some degree of degranulation. A few thyroidectomy cells could be found in these glands. In addition to the numerous well-granulated large Gomori positive cells in these glands, there were many small cells which contained a thin rim of fine granules around the nucleus (Figure 8). The degree of regional predilection of the Gomori-positive cells is not evident in the pituitaries at this stage (Figure 10). Concentration in the central portion of the anterior lobe was no more common than in other parts or regions of the gland. The control gland at this stage shows a concentration of the thyrotrophs in the central portion of the anterior lobe (Figure 9).

Sections of the pituitary glands which were removed from the experimental animals following thiouracil administration for a period of 18 months (Figure 4) showed, in most cases, a progressive degranulation of the thyrotrophs present and also a generalized thinning out and disappearance of this type of basophil throughout the entire gland. At this stage there were also a few scattered Gomori-positive cells having a rim of fine granules around the nucleus. There was no increase in the number of thyroidectomy cells in the glands at this stage over that found in the 12 months series. Figure 7 shows the presence of Gomori-positive cells from the densely stained type through the various stages of degranulation as well as a thyroidectomy cell. This figure also illustrates how the thyrotrophs may be arranged in immediate proximity to the various blood vascular channels as well.

FIGURE 1

Anterior pituitary of the control rat sacrificed six months after initiation of thiouracil administration to the experimental animals. The glands removed from the control rats six months later were no different from these. The region of the intermediate lobe is located in the upper right corner where the residual lumen can be seen. Aldehyde-fuchsin, X 70.

FIGURE 2

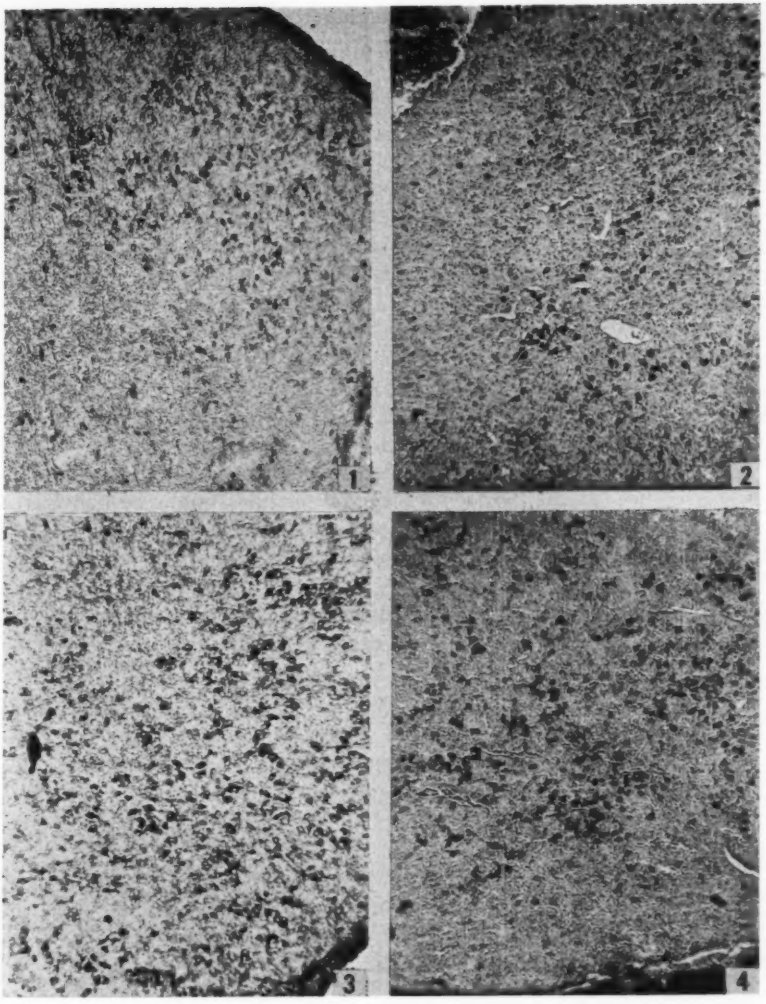
Anterior pituitary after six months of thiouracil administration. Note thinly scattered thyrotrophs throughout the section. Intermediate lobe shown in upper right corner. Aldehyde-fuchsin, X 70.

FIGURE 3

Anterior pituitary following 12 months of thiouracil administration. Thyrotrophs uniformly scattered and more densely stained. Note many small cells containing a fine rim of granules around the nucleus. Intermediate lobe region shown in lower right corner. Aldehyde-fuchsin, X 70.

FIGURE 4

Anterior pituitary after 18 months of thiouracil administration. Degranulation of many of the thyrotrophs is evident. No thyroidectomy cells are found. Note the increased vascularization. Intermediate lobe in lower right corner. Aldehyde-fuchsin, X 70.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While the weights of the pituitaries failed to increase greatly over that of the 12 month series, the glands seemed to be enlarged and histologically showed evidence of a continued increase in vascularization. The control glands at this stage appeared to be the same as at the 24 month period (Figure 5).

Twenty-four months after the initiation of the experiment the pituitaries of the remaining experimental animals had increased in weight approximately 30 per cent over the controls, and a progressive increase in vascularization was very much in evidence at this time. Of the 29 experimental animals sacrificed at this time, nineteen of them had pituitary glands which failed to exhibit any thyrotrophs with the aldehyde-fuchsin stain (Figure 6). The pituitaries from the ten remaining rats of this twenty-four month period showed nodular groups of cells in the anterior lobe. These nodules varied in number from one to three in each gland. In all but one of these nodular glands the cells making up most of the nodule reacted to the aldehyde-fuchsin stain positively (Figure 12). In the one gland the nodules failed to stain with either the aldehyde-fuchsin or the periodic acid-Schiff stain. On this basis the pituitary nodules might be considered basophilic adenomas, with the exception of the one gland where the adenoma could be called a chromophobic adenoma. In no case were the adenomatous glands any larger in size or in weight than the non-adenomatous glands in the experimental series. In only one of the 5 normal control animals sacrificed at 24 months was there evidence of nodular formation and in this case the nodular cells failed to stain positively with the Gomori-aldehyde fuchsin stain or the periodic acid-Schiff stain (Figure 11).

Discussion

There is substantial evidence that the thyrotrophs (Purvis and Griesbach 1951a), also called beta cells by Halmi (1950, 1952), liberate thyrotrophin from the anterior pituitary in the rat and also in the mouse (Halmi and Gude, 1954). The above investigators and others have presented data to support this concept on the basis of these cells giving rise to the so-called thyroidectomy cells under conditions of thyroid hormone deficiencies and also a regression of these same cells when under the influence of hyperthyroid conditions.

With the administration of thiouracil to rats for prolonged periods, it is well known that thyroid hormone levels will be reduced and a

concurrent increase in the secretion of anterior pituitary thyrotrophin is to be expected. In our series of experiments it was of interest to administer thiouracil for extended periods to note whether compensatory hypertrophy and eventually exhaustion atrophy would result. The results of these experiments appear to indicate that hyperplasia and hypertrophy of the thyrotrophs do initially occur. The hyperplasia may be initiated through those beta cells which are outlined with granules and which are also reported as occurring in the rat the first day after birth (Siperstein et al., 1954). Our results also confirm the work of Seifter, Ehrich and Hudyma, 1940, in regard to the absence of thyroidectomy cells in rats which had been given thiouracil for more than one year. It is possible that the absence of thyroidectomy cells in the later periods of our experiments may be the result of transitional basophilic changes somewhat similar to those described by Wilson and Ezrin (1954). In their experiments the various degrees of suprarenal activity were thought to influence the basophilic staining reactions. However the significance and correlation of these reactions must await further investigative studies.

The failure to find any great number of cells which stain with the aldehyde-fuchsin stain after two years of antithyroid administration to these rats does not necessarily indicate that these beta cells are not to be found in the glands of these animals. Assays of the glands for thyrotrophin content may still produce evidence of high hormone concentration in spite of the lack of staining ability of the specific cells involved. Further experiments are also being carried out on a series of these thiouracil rats where the drug is discontinued after 24 months and thyroxin administration is substituted to note whether the thyrotrophs will reappear when stained with aldehyde-fuchsin.

The cytological and physiological stimulation of the pituitary following the administration of thiouracil or other antithyroid drugs is now well known. It is possible that some functional stress is thus placed on the pituitary and that a prolonged period of this stress may be sufficient to result in a tumorous condition in the pituitaries of these animals. The incidence of nodule formation in the pituitaries of our experimental series (65 per cent) cannot be attributed to the relatively high spontaneous incidence of hypophseal tumors in rats of this age. This would appear to be especially true when the low rate of tumor incidence (20 per cent) in our control series of experiments is considered. While the presence of pituitary tumors in rats treated

FIGURE 5

Section of control pituitary taken from rat sacrificed 24 months after initiation of the experiment. The glands removed from control rats six months earlier were the same as this. Note intermediate lobe in lower left corner. Aldehyde-fuchsin, X 70.

FIGURE 6

Anterior pituitary after 24 months of thiouracil administration. Almost complete absence of the Gomori positive cells. Note the increased vascularization of the gland. Aldehyde-fuchsin, X 70.

FIGURE 7

The Gomori positive cells of the anterior pituitary. The various degrees of degranulation can be noted as well as the close proximity of these cells to the blood channels. A thyroidectomy cell can be seen near upper left border. Aldehyde-fuchsin, X 850.

FIGURE 8

Gomori positive beta cells of anterior pituitary gland of rat subjected to 12 months of thiouracil. These cells with a fine rim of granules near the cell periphery are extremely numerous in the hyperplastic glands. Aldehyde-fuchsin, X 850.

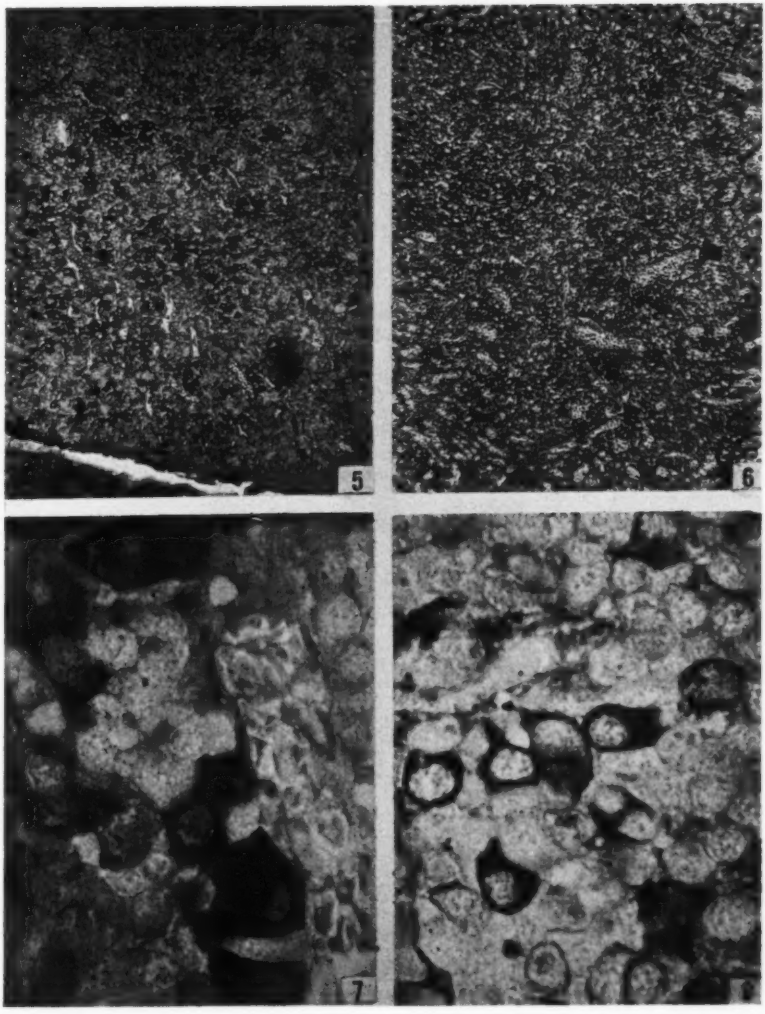
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FIGURE 9

Section of control pituitary removed 12 months after initiation of the experiment. Note general tendency of Gomori-positive cells to be concentrated in more central portion of the gland. Aldehyde-fuchsin, X 36.

FIGURE 10

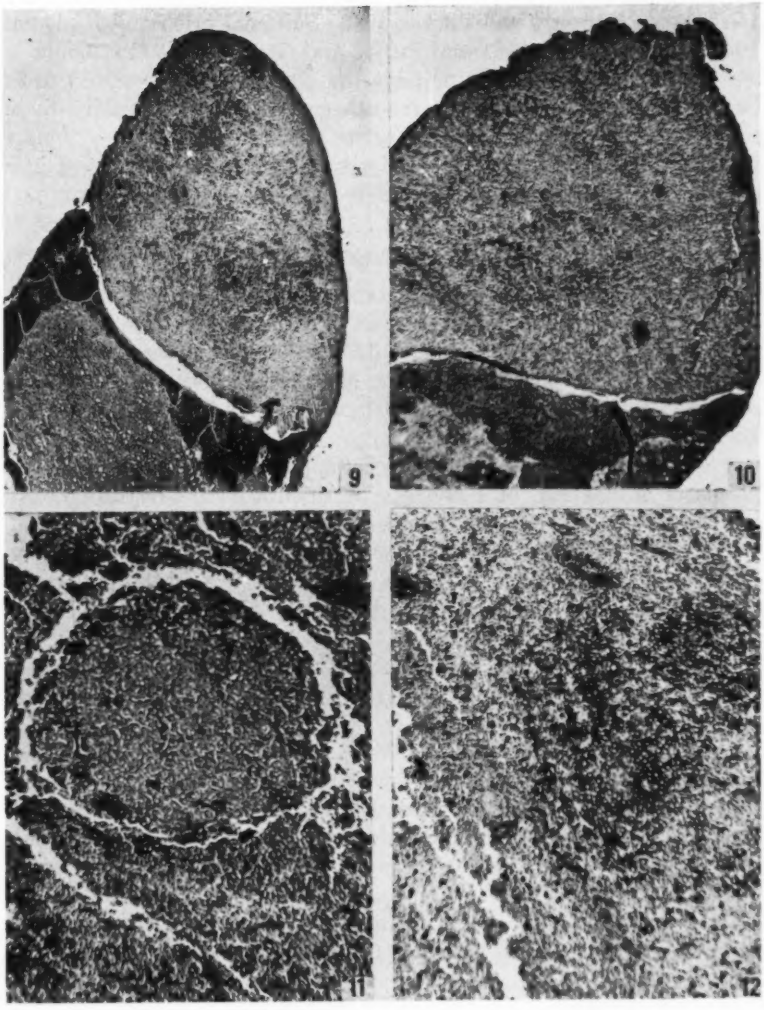
Section of anterior lobe of pituitary from a rat subjected to thiouracil for 12 months. Note the peripheral as well as the central distribution of the thyrotrophs. Aldehyde-fuchsin, X 36.

FIGURE 11

Section of anterior lobe of pituitary of a control animal sacrificed at termination of experiment. This nodular adenoma failed to show thyrotrophs when previously stained with Aldehyde-fuchsin as well as with this stain. Periodic acid-Schiff, X 70.

FIGURE 12

Section of anterior lobe of pituitary after 24 months on thiouracil. The Gomori positive cells make up the majority of the cells in the nodular adenoma of this gland. Aldehyde-fuchsin, X 70.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with goitrogens for prolonged periods has been frequently reported (Greisbach, Kennedy and Purvis, 1945; Seifter, Ehrich and Hudyma, 1949; Sellers, et al., 1953 and Purvis and Greisbach 1951a). Only in the last mentioned publication have these tumors been referred to as basophilic adenomas. Most of the other authors have classified these tumors as chromophobic. Since the majority of the pituitary tumors in our experiments reacted to Gomori's aldehyde-fuchsin reagent in a positive manner, it seemed appropriate that these adenomas be termed basophilic adenomas.

Summary

The anterior lobes of pituitaries of male rats were studied histologically following the administration of thiouracil to a series of animals for periods of 6, 12, 18, and 24 months. All of the glands were sectioned serially and stained with aldehyde-fuchsin reagent to observe the thyrotrophic basophils. Representative findings are as follows:

1. The beta cells or thyrotrophs appeared to be undergoing hyperplasia at the end of 6 months of thiouracil administration. No hypertrophy of the cellular components was evident.

2. At 12 months the beta cell granules stained more densely. Some evidence of degranulation was present. A few scattered thyroidectomy cells were present. Many beta cells with only a thin rim of cytoplasmic granules were found.

3. Eighteen months of thiouracil administration produced beta cells which showed extensive degranulation. Increased vascularization of the glands was noted.

4. After 24 months of thiouracil administration the glands had increased in weight 30 per cent over the controls. Very few Gomori positive cells were to be found in the anterior lobes at this time. Increased vascularization was extensive and most evident at this time.

5. Many nodular formations were to be found in a number of the glands of these rats after two years of thiouracil administration. The nodules were classified as basophilic adenomas on the basis of their staining reactions to the aldehyde-fuchsin stains.

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BOOK REVIEW

Symposium on Genetic Recombination. Sponsored by The Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. April 19-21, 1954. Journal of Cellular and Comparative Physiology *45*, Supplement 2, May 1955. 316 pages.

To the reader who wishes to review present trends in genetics this volume is heartily recommended. Two fields are dealt with, genetic recombination and the oxygen effect on radiation-induced chromosome alteration. Of ten papers in the former field four deal with bacteria and phage, five with recombination phenomena in species where chromosome analysis is possible, and one (by Watson) with the biological consequences of the complementary structure of DNA. Each is well written and is followed by discussion.

Of special interest are those dealing with recombination in microorganisms. Doermann, Chase and Stahl describe convincingly genetic recombination and replication in bacteriophage, with the classical concepts of these terms. Lederberg calls the genetic phenomenon peculiar to bacteria, transduction, and defines it as the transmission of a (nuclear) genetic fragment from a donor cell to a recipient cell. He distinguishes two agencies of transfer, deoxyrimonucleic acid (DNA) in an extract of other bacteria and virus particles (phage). He likewise discusses recombination mechanisms in bacteria which parallel those in higher organisms. Zinder restricts the term transduction to transmission of genetic fragments by phage in his exhaustive treatment of this subject. Hotchkiss writes of the latest progress in study of transformation induced in bacteria by DNA. He concludes that "bacterial transformations are focussing attention on the critical genetic processes that go on in cell divison, probably no less important than the events of cell fusion, already so well documented". The question asked by Lindgren as to why there is no evidence of transduction or transformation in hybrid bacteria remains unanswered.

Perkins brings together data from widely different organisms and discusses details of tetrad behavior in relation to recombination. Novitski uses the behavior of abnormal chromosomes such as dicentrics in Drosophila for establishing concepts of centromere activity while, from studies on crossing over in maize and Drosophila, Schwartz suggests that chromosome duplication involves the formation of a new chromatid on a template provided by the parent chro-

mosome as proposed by Belling in 1931. He discusses somatic and meiotic crossing over and presents arguments favoring sister strand crossing over. Whittinghill summarizes the influence of external factors such as X and γ rays on induction and on rates of crossing over and presents information on stages in which crossing over may occur. Variation in genetic recombination in natural populations is discussed by Carson who finds that marginal populations are genetically more flexible than centrally located ones in capacity for immediate adjustment to new conditions.

The three scientists evaluating these papers have long been associated with classical cytology and genetics. Sturtevant questions some of the theories of Lederberg and the recent tendency to throw out the classical theory of the gene as a discrete unit. Sax states that "as an old-fashioned cytologist I should like to present certain aspects of chromosome structure and behavior which must be considered in any evaluation of the recombination theory." This he proceeds to do. Weinstein, the third evaluator, gives a series of diagrams and descriptions of the possible methods of chromosome behavior, the phenomena to be explained and the possibilities of explanation, a scholarly presentation by an authority.

The three papers dealing with the oxygen effect on radiation-induced chromosome aberrations, breakage-versus-recombination hypotheses, by Giles, by Swanson and by Baker and von Halle, and the discussion by Conger summarize the arguments on both sides.

We have in this symposium, the healthy situation of a combination of ideas of enthusiastic young investigators studying new genetic phenomena and devising theories to explain them and of those of experienced geneticists and cytologists unwilling to abandon older theories until convincing evidence against them is presented but, at the same time, objectively interested. Genetics remains an active, growing division of natural science.

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